

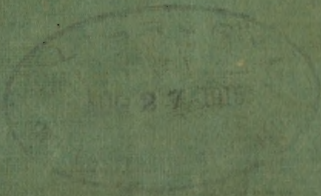
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COLLECTED PAPERS

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DETROIT, MICH.

DR. E. M. HOUGHTON, Director.

Reprints—Volume 3
1915



Biological
& Medical
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TIME RECORDER FOR KYMOGRAPH TRACINGS.

OLIVER E. CLOSSON.

(From the Research Laboratory, Parke, Davis & Co., Detroit, Mich.)

Received for publication, November 1, 1913.

In all graphic records of phenomena the time relations are of prime importance. Records made upon smoked paper are especially subject to detriment if measurements are attempted before the soot has been fixed, and even then it is a tedious and often unsatisfactory operation to find from the time curve the projection of the time intervals on the different tracings.

The apparatus here described records the time interval by a fine line entirely across the smoked paper, so that the time relations are everywhere apparent. The record thus appears with the tracings crossed by a series of parallel lines which represent the lapse of time for which the clock is set.

The cut, A, shows lines at two second intervals. The upper tracing is peristalsis of the small intestines recorded by means of a balloon in the lumen and air transmission to a tambour. The middle curve is the blood pressure and pulse at the carotid recorded by a float in a mercury manometer. The base line records by a down stroke the intrafemoral injection of the dog with a six months old preparation of secretin and by the up-stroke the drops of pancreatic juice secreted.

The principle of the device is exemplified when the artisan snaps a chalked cord for drawing a straight line between two points; thus if a fine wire is stretched parallel to the smoked paper at a distance of 2 or 3 mm., it will, when pulled away and then released, snap back and remove a fine line of soot from the paper.

The accompanying drawing B and photograph C show the essentials of the apparatus. An iron bar *B* has both ends bent at right angles to the main portion, and slits cut in the ends with a hack-saw, as shown. This forms a bow and a fine spring wire is stretched between the ends. A signal magnet *M* is attached so that the pick *P* on the extended armature plucks the wire when

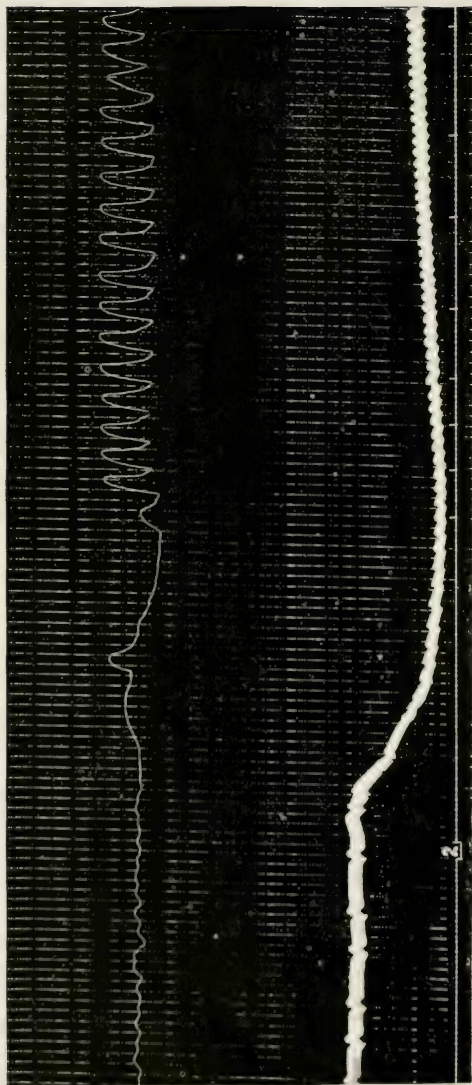


FIG. A.

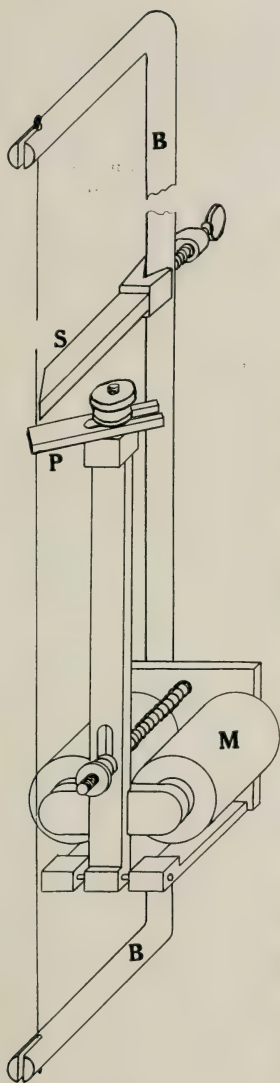


FIG. B.

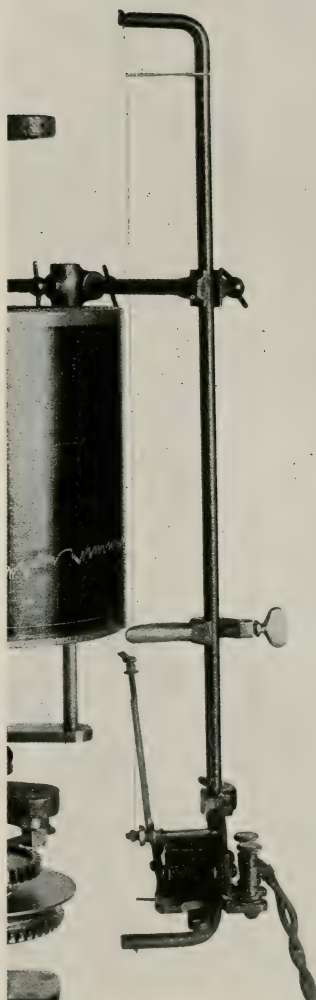


FIG. C.

the armature is attracted at the passage of an electric current. By a little adjustment it can be made to pull the wire the full excursion before releasing and to slip past the wire without trouble on returning to position. This action can be made very certain by attaching an inclined stop *S* set to push the wire off the pick at any given point in the throw. All irregularities are thus avoided even when the pick is obviously set improperly. The signal magnet is connected with the time clock in the usual way, and the apparatus clamped before the drum so that the spring wire will record a single line at the rebound from each pull of the signal magnet.

When the wire is stretched too tight there is often recorded a double line for a part of the distance especially on wide paper; however, by damping the vibrations this is entirely avoided. A small rubber band around the wire and bow damps the vibrations sufficiently.

The portion of the wire above the signal magnet is used for writing, and by having the bow 2 feet long, there is no trouble in recording on 10-inch paper. If it is inconvenient to adjust any recorder to write perpendicular to the base line, it is a simple matter to adjust so that the time line is parallel to any such line. This is shown in the cuts where the time lines do not make right angles with the base line.

U. S. P. 1900 MENSTRUUA.

BY H. C. HAMILTON.

(From the Research Laboratory of Parke, Davis & Company, Detroit, Mich.)

It seems almost superfluous to call attention, at this late date, to certain points in the 8th Revision of the U. S. P. which need correction in the forthcoming 9th Revision. Particularly does it seem unnecessary in view of the fact that the objectionable features to which this article alludes have been pointed out before and by several critics. The excuse for doing so, however, if any is necessary, is that the data here published may be of value to those who have under consideration for the 9th Revision of the Pharmacopœia the menstrua for the extraction of the digitalis series of heart tonics. The menstrua to which we refer are for the preparation of: I, F. E. Digitalis; II, F. E. Squill; III, F. E. Convallaria.

I. The first two of these were referred to by Houghton and Hamilton¹ in the following words:

"3. Fluidextract digitalis, U. S. P. 8th Rev., 48 per cent. alcohol.

"Average potency of eleven samples at time of manufacture 55 H. T. U. per c.c. Three and a half years later 35 H. T. U. Average loss about 10 per cent. yearly.

"A very important point should be noted in this connection, namely, the menstruum adopted in the last U. S. P. for the preparation of fluidextract digitalis is much less desirable than the U. S. P. 7th Revision in at least two respects. Repeated trials show that it is almost impossible to get a finished product containing the full number of H. T. U. of the standard we had previously adopted, the average being as above stated, 55 H. T. U. per c.c., while with the drug of the same quality when the 7th Revision menstruum is employed no difficulty is experienced. Owing to this it was decided to no longer attempt to assay physiologically the 8th Revision product and to take such statement referring to it off the label, but, in order to sup-

ply the medical profession with a full strength fluidextract of the drug, it was decided to prepare such with a menstruum containing a larger per cent. of alcohol which could be assayed and so labeled. In the second place the loss in potency of the 8th Revision is about 10 per cent. per year, while with the 7th Revision it is less than one-half as great, or about 4 per cent. The results coincide quite closely with those following the change made in the menstruum for the fluidextract of squill, except that the loss in activity was greater in the latter drug, as pointed out by Houghton² three years ago. In this paper several methods of physiological assay showed very clearly that a serious mistake had been made in changing to acetic acid as a menstruum. The writers feel certain that any one who has tried the 8th Revision menstruum for fluidextract digitalis has found that it is much less satisfactory from a pharmaceutical point of view, to say nothing of the loss in potency."

To this we wish to add data since obtained on F. E. Digitalis as follows:

Menstruum.	Per cent. Activity.
50 per cent. alcohol.....	100
80 per cent. alcohol.....	120

The above samples were prepared from one lot of drug, using 100 grams and extracting until exhausted.

Another small sample of drug carefully extracted by both methods and tested gave results as follows:

with 50 per cent. alcohol.....	110 per cent. of standard.
with 80 per cent. alcohol.....	140 per cent. of standard.

A sample of drug extracted with several strengths of alcohol gave the following results:

Menstruum.	Per cent. Activity.
94 per cent. alcohol.....	90
75 per cent. alcohol.....	140
62.7 per cent. alcohol.....	125
50 per cent. alcohol.....	110

The following table shows the tests of commercial lots of F. E. Digitalis, U. S. P. 8th Rev. (*a*) before and (*b*) after an attempt to improve the quality by concentrating the extract.

Number.	Tested.	Per cent. Activity.
1 (a)	8/4/9	85
1 (b)	8/19/9	90
2 (a)	7/20/9	85
2 (b)	8/4/9	85
3 (a)	3/4/9	60
3 (b)	4/2/9	100
4 (a)	1/31/8	80
4 (b)	2/8/8	80
5 (a)	5/23/7	75
5 (b)	6/1/7	83

Further data on 20 samples of the preparation show results of first tests ranging from 50 to 100 per cent. standard and averaging exactly 78 per cent.

The standard referred to is the average activity obtained from 12 lots of crude drug, botanically of first class quality, selected at random and extracted with 62.7 per cent. alcohol, the official menstruum of the U. S. P. 7th Revision. The activity was determined by the frog method described by Houghton³ as a means of standardizing the heart tonics of the digitalis series. In that article attention was called to the enormous variation in samples of the crude drug for sale on the open market.

The value of such a method is also shown when endeavoring to extract from active material all the therapeutically active substances and to establish by experiments on other than the human subject the relative activity of extracts obtained by means of various menstrua.

The above results speak for themselves, but if additional authority is needed it should be sufficient to note that the menstrua for making tinctures and fluidextracts of digitalis in the official Pharmacopœiæ of the world, specify, almost without exception, a percentage of alcohol in excess of that official in the U. S. P. 8th Revision. The menstruum adopted in 1906 by the Brussels Conference⁴ is 70 per cent. alcohol and it is to be hoped that the Revision Committee will be influenced by this in adopting an official menstruum for the 9th Rev. of the U. S. P.

II. As noted before in the abstract from the AMERICAN JOURNAL OF PHARMACY¹ a mistake was certainly made in adopting for the preparation of F. E. Squill, U. S. P., 8th Rev., a menstruum composed of a 10 per cent. solution of Acetic Acid.

This is so far from being ideal for extracting the active substances from Squill bulb that it is practically impossible to prepare an extract representing the activity of the crude drug.

Comparison of the activity of F. E. Squill, U. S. P., 1890 and 1900, was made by Houghton⁵ as follows:

"Comparative Strength of Fluid Extract of Squill Prepared from the Same Lot of Drug According to the United States Pharmacopœia of 1890 and 1900:

"1 U.S.P., 1890, 140 per cent. as active as standard fluid extract.

"2 U.S.P., 1890, 140 per cent. as active as standard fluid extract.

"3 U.S.P., 1900, 60 per cent. as active as standard fluid extract.

"4 U.S.P., 1900, 60 per cent. as active as standard fluid extract.

"It may be observed that activity of both products is high as compared with the results given in Table 2. This probably is due to the great care exercised completely to exhaust the drug and to the high quality of the drug.

"In order to meet any objections that might be offered against the results as shown by the special method of assay employed, the work was checked by experiments on dogs showing the comparative activity of the two products in producing changes in the blood-pressure, which is perhaps the most characteristic physiologic action of the members of the digitalis series."

The results of the latter experiments are here recorded in tabular form for more convenient reference.

EXPERIMENT I.

	F. E. Squill, U. S. P., 1890.		F. E. Squill, U. S. P., 1900.	
	Before injection.	After injection.	Before.	After.
Pulse Rate	100	96	116	138
Blood-pressure	46	54	48	45

In this experiment 0.3 c.c. F. E. Squill, U. S. P., 1890, was injected at 10.15 A.M. into the femoral vein of an anesthetized dog. Then at 2.11 P.M., when the effect of the first injection had passed, the same amount of F. E. Squill, U. S. P., 1900, was injected.

In the second experiment the order of injection was reversed, another dog being used for the test, and the same amount of each preparation injected.

EXPERIMENT II.

	F. E. Squill, U. S. P., 1900.		F. E. Squill, U. S. P., 1890.	
	Before.	After.	Before.	After.
Pulse Rate.....	102	144	100	94
Blood-pressure	47	46	52	50

NOTE.—In both cases the U. S. P., 1900, preparation increased the rate and lowered the pressure. This is directly opposite in effect from the characteristic action of the heart tonics in general and from that of the F. E. Squill, U. S. P., 1890, from the same drug.

In this case again a stronger alcohol is better. If the drug is finely ground and extracted with menstrua containing 60 per cent. or less of alcohol, it swells so that percolation is either entirely or almost prevented. It becomes necessary either to cut the bulb without grinding or to mix with sawdust in order to have it sufficiently open to percolate properly. An additional objection is in the large amount of gummy, water-soluble extractive obtained with such menstrua. A fluid extract of better appearance, better keeping quality and containing practically all the available activity of the drug, can be obtained by the use of 80 per cent. alcohol. Repeated experiments have shown the excellence of this menstruum over that of the 7th or 8th Revisions of the U. S. P.

III. Fluid Extract Convallaria, U. S. P., 1900, is not so open to criticism as the others, but the menstruum is not entirely satisfactory. There are certain advantages to be gained by using a stronger alcoholic menstruum than that prescribed in the 8th Revision U. S. P. While these advantages are more apparent when experiments are conducted on a manufacturing scale than when small experimental lots of fluid extract are prepared, even in the latter case the advantages are very real.

Several experiments have been carried out, of which the following is used as an example:

A small lot of drug was divided into two portions, one of which was extracted as prescribed in the U. S. P., namely, with 62.7 per cent. alcohol, the other with 80 per cent. alcohol. These extracts were carefully concentrated to fluid extract volume and tested for activity by the method previously cited, with the following results:

Menstruum.	Per cent. Activity.
62 per cent. alcohol	100
80 per cent. alcohol	120

The advantages to be gained from using a stronger alcoholic menstruum for extracting cavallaria roots and rhizome are not merely the greater activity obtainable, but in the improved appearance of the extract and its greater stability. It contains less of the gummy extractives and more alcohol, both of which are desirable features, as they affect deterioration, while the 20 per cent. increase in activity from the use of 80 per cent. alcohol is no less desirable.

It is to be hoped that those in charge of revising the forthcoming U. S. Pharmacopœia will consider these suggestions.

LITERATURE CITED.

- American Journal of Pharmacy*, October, 1909.
Trans. American Medical Ass'n., June 12, 1906.
Ibid., September 11, 1897.
 Oscar Oldberg, "Pharmacy," pp. 57-66.
Trans. American Medical Ass'n., May 12, 1906.

NUMERICAL VARIATIONS OF THE WHITE BLOOD CELLS IN MICE INOCULATED WITH TRANSPLANTABLE ADENOCARCINOMA.

BY F. W. BAESLACK, M.A., M.D.

(From the Research Laboratory, Parke, Davis & Co., Detroit, Michigan.)

With 19 figures in text.

(Eingegangen bei der Redaktion am 27. Oktober 1913.)

The exchange of metabolic products within the organism is to a large extent affected by the blood and lymph, so that both may be looked upon as the transmitters of ferments and their products from tissue to tissue, and any changes from the usually prevailing conditions have in turn their influence upon the cells of the blood, so that, from the picture which the blood presents, we are able to gauge the course of certain diseases.

Many of the acute infectious diseases, as well as those which have severe chronic disturbances of nutrition and metabolism in common, have their influence upon the blood. Thus every process of infection and intoxication is to a certain degree a picture of the destruction of the white cells, peculiar to that process. This destruction is in proportion to the severity of the infection or intoxication; to the specific action of the infecting or intoxicating agent on the blood, and blood forming organs; and to the resistance of the organism.

The observation that the number of the white blood corpuscles was frequently increased in cancer has been made by numerous observers. Andrae (1) was perhaps the first who recognized the leucocytes in the abnormally increased number of cells. The findings of Andrae were confirmed by Vidal (2) and a number of other French observers. More detailed observations on this subject were made by Lücke (3), who concluded that this increase in the number of leucocytes in cancer was a sign that the disease had become general and involved the entire body. Neubert (4) was unable to find any cardinal changes in the number of leucocytes. He noted, however, that the numerical relationship between the mononuclear and polynuclear forms of white blood cells had been shifted in favor of the polynuclear cells. Very exact data concerning the occurrence of leucocytosis in cancer are found in the observations of Hayem and Alexander (5). These authors noted that not all cases of carcinoma and sarcoma of various organs were accompanied by a leucocytosis. In ten of the fourteen cases of scirrhus growths, and in five out

of twelve cases of cancer of the stomach a leucocytosis was observed. The relationship which possibly exists between the seat of the tumor and its histological structure is not considered by them. Schneider (6) cites twelve cases of cancer of the stomach, in all of which leucocytosis was present, while Laache (7) was unable to find any in the five cases he reported. In a series of forty-six cases of cancer of the stomach cited by Cabot (8), fifteen showed leucocytosis and thirty-one showed none. "In some of the cases the counts were verified by repeated examinations, while in others only a single count, that made when the patient entered the hospital, was recorded." An equal divergence of opinion exists in regard to the leucocyte count in cases where the malignant new growth involved other organs. Thus Hayem was unable to note an increase in the number of leucocytes in 6 cases of cancer of the uterus, whereas Rieder found 30,800 in a single case, and Cabot cited three cases each showing distinct leucocytosis. One may conclude from these reports that leucocytosis is the rule in carcinoma, but that there are many exceptions, and that the leucocytosis is in proportion to the malignancy, to the rapidity and the extent of the growth of the tumor. Factors which govern the degree of leucocytosis seem to be the position of the tumor, the resistance of the individual, the daily variations in the growth of the tumor as well as the liberation of the metabolic products from it, which carried by the blood stream may influence the blood-forming organs.

The location of the tumor determines to a considerable degree the facility with which the products of metabolism of the cancer cell enter the blood or lymph stream. Enzymes, liberated through the breaking down of the tumor cells, find their way into the circulation and stimulate the formation of other ferments in the organism. While cancer tends to increase in size, invading the surrounding tissues, its rate of growth is not uniform, periods of slow and rapid growth alternate with periods during which growth seems to be entirely arrested, or we may even note retrogression of the tumor in certain parts with rapid development in others at the same time. Furthermore, on account of the abnormal metabolism of the cancer cell, products of this metabolic action are discharged into the blood, which may to a large extent cause an increase in certain white blood cells. This possibility has been advanced by Meltzer (9), who observed that there is a difference in the ferment between the leucocytes and the lymphocytes, since the one acts in alkaline, the other in acid media, and that there might be some definite relation between certain conditions of acidity and lymphocytosis.

In the histological study of retrograding tumors in mice (10) one is impressed by the large amount of round cell infiltration at the margin of the tumor and along the strands of actively proliferating connective tissue, which may be observed dipping into the tumor undergoing retrogression. This preponderance of small round cells about the margin of such tumors has also been observed by von Hanseman, Wisnieski and recently again by Da Fano (11), who points out that the development of tumor immunity is coincident with a general reaction of the connective tissue throughout the organism. The polynuclear leucocytes appear first at the place of the implanted tumor, where under aseptic conditions, without showing phagocytic activity, they undergo degeneration. The lymphocytes then appear in large numbers around the inoculated tumor to diminish again gradually after immunity is established. That the leucocytes seem to be in a close relationship to the development of tumor immunity is, furthermore, shown by the fact that the inoculation of dead tumor cells is followed neither by this round cell infiltration nor by the development of tumor immunity.

To determine whether the growing or retrograding neoplasm had any influence on the ratio of the white blood cells, total as well as differential counts were made in mice inoculated with transplantable tumors, and on two spontaneous tumor mice found among our own stock in the course of these experiments. A total as well as differential blood count was made on each mouse before inoculation. The blood for the succeeding daily differential counts was taken each morning, and was obtained by cutting off a small portion of the tail of the animal. The second or third drop of blood was used for making the blood smear. The wound was sealed by a cautery to prevent infection. The smears were stained with the Wright's modification of Jenner's stain. In the early experiments 500 cells were counted from each preparation, later 200 cells. The cells counted were the polymorphonuclear leucocytes, small mononuclear lymphocytes, large lymphocytes and eosinophiles. The counts from day to day were tabulated and the percentages of the polymorphonuclear leucocytes and small lymphocytes were plotted. The large lymphocytes and eosinophiles were not included in the charts to avoid making them too complicated.

The number of mice used in the individual experiments was small, because a small number of animals could be kept under better observation, and it was thought best to allow a long period of time for this work, so that the effect of the variation in the

virulence of the tumor might be noted. In all about 50 mice served as a basis for these experiments, which extended over the period of one year. The tumors used for transplantation were tested in each instance for sterility by means of smears and cultures, as were also a large number of tumors of these mice at the time of their death, for the purpose of excluding any error of variation in the blood counts due to terminal infection. The tumors were charted every other day to note any change in their size and rate of growth.

From the experiments forming the basis for this communication, the records of four have been selected as representative of the possible fate of the transplanted tumor material. In the first experiment we have a non-take, in the second a rapid tumor development following inoculation, in the third is brought out spontaneous retrogression, while the fourth experiment permits of comparing the changes of the differential blood count with the actual blood counts on the dates indicated.

The differential blood counts done on the spontaneous tumor mice do not vary from those observed in inoculated mice.

Experiment I. Serial No. 22. May 17, 1911.

Mouse tumors 20 F and G employed for inoculation. Weight of mice 16.5 gr. and 17.5 gr. respectively. Weight of tumors 1.1 gr. and 2.1 gr. respectively. Age of tumors (time elapsed from date of inoculation to May 17th), 9 days.

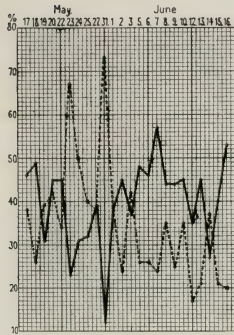
Ratio of tumor to salt solution 1:3, dose 0.5 ccm.

Two mice were inoculated and were kept under observation from May 17th until June 17th, 1911. No tumor developed, although the lot from which these tumors were taken gave a yield of 77%.

Both charts exhibit a fall of the small mononuclear lymphocytes and a rise of the polymorphonuclear leucocytes. This fall in the number of the mononuclear lymphocytes is noticeable at once, following the inoculation, and the divergence in the qualitative ratio of these two blood constituents increases from 10—13 days after inoculation, to be followed by a rise in the small lymphocytes and a drop in the polymorphonuclear leucocytes. The length of time required for the absorption of the inoculated tumor material is about the same, while the rate of absorption is

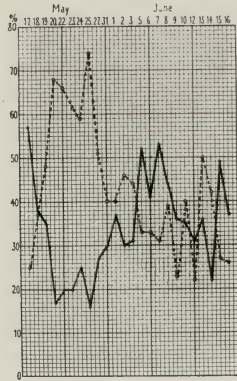
not uniform, as appears in the chart of 22A from May 23rd to 31; and from May 20th to 25th in the chart of mouse 22B.

Fig. 1.



— Small Mononuclear Lymphocytes
.... Polymorph. Leucocytes

Fig. 2.



— Small Mononuclear Lymphocytes
.... Polymorph. Leucocytes

The mice happened to be immune to this strain of tumor, for no tumor developed after reinoculation on June 18th.

Experiment II. Serial No. 38. June 27, 1911. (See Plate I, p. 19.)

Mouse tumor 32 D employed for inoculation. Weight of mouse 28.5 gr. Weight of tumor 5.6 gr. Age of tumor (time elapsed from date of inoculation to June 27th, 1911), 25 days.

Ratio of tumor to salt solution 1:3; dose 0.5 ccm.

Three mice were inoculated.

Letter	Died	Weight of		Remarks
		Animal	Tumor	
A	7/10/11	22.0	Large tumor; about 8.0 gr.
21 0 gr. B	7/20/11	26.1	8.55
21.0 gr. C	7/22/11	25.6	8.61
24 0 gr.				

As appears from the above records, all three mice developed large tumors which grew very rapidly, killing the mice in 13, 23 and 25 days respectively. Mouse A was decomposed, so that it was impossible to perform an autopsy. Mice B and C were autopsied and cultures were made from the tumor and liver on agar-agar and bouillon which were sterile after 24 hours' incubation.

Fig. 3.

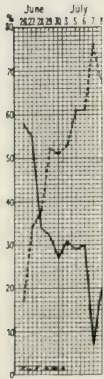


Fig. 4.

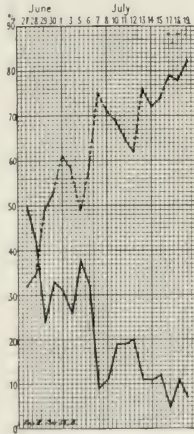
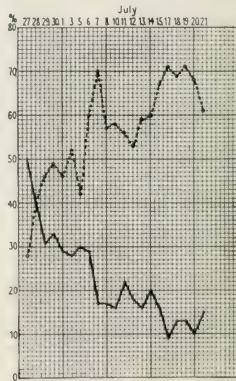


Fig. 5.



— Small Mononuclear Lymphocytes
 Polymorph. Leucocytes

— Small Mononuclear Lymphocytes
 Polymorph. Leucocytes

As in charts 1 and 2, the above charts exhibit the same decrease in the mononuclear lymphocytes and the increase in the polymorphonuclear leucocytes following the inoculation. However, the lines continue to diverge as the tumor cells injected begin to multiply, giving rise to a tumor weighing at least eight grams in each of the three mice inoculated. That the decrease in the small mononuclear lymphocytes is not relative, but actual, appears from the blood count made on mouse C the day before it died. The total number of red cells had fallen from 10,440,000 the day before inoculation to 2,528,000; and the total

number of white blood cells had risen from 14,500 to 24,920. The number of polymorphonuclear leucocytes the day before the inoculation was 123 out of 438 cells counted, or 28.08%, and that of the small lymphocytes was 220, or 50.22%; while the day before the animal died the number of polymorphonuclear leucocytes was 327 out of 532 cells counted, or 61.46%, and that of the small lymphocytes was 83, or 15.6%. For the charting of the tumors see Plate I, p. 19.

Experiment III. Serial No. 49. August 17, 1911. (See Plate II, p. 19.)

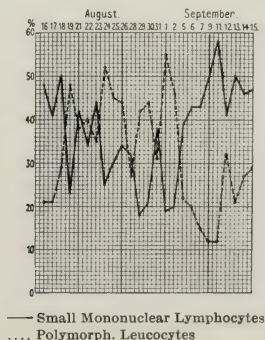
Mouse tumor employed: 41 B. Weight of mouse: 25.0 gr. Weight of tumor: 4.0 gr.

Age of tumor from inoculation to death: 31 days.

Ratio of tumor to salt solution 1:3. Dose: 0.5 ccm.

Letter	Died	Weight of		Remarks
		Animal	Tumor	
A 25 gr.	Last charting 11/14/11. Tumor was absorbed. Released 11/14/11.
B 23.0 gr.	10/21/11	22.0	7.3
C 26.0 gr.	Recovered. Released 11/14/11.
D 18.0 gr.	9/9/11	19.0	6.0

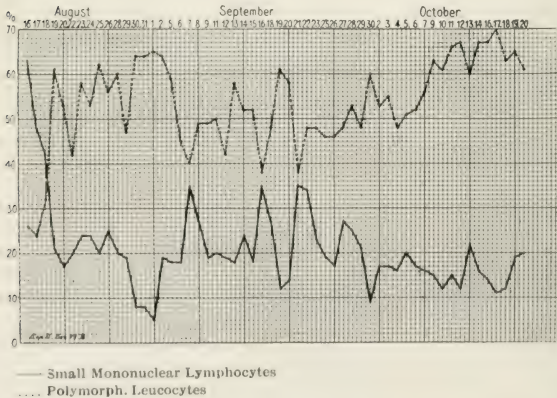
Fig. 6.



As pointed out in the two preceding experiments, there is an initial rise in the number of polymorphonuclear leucocytes and a fall in the small mononuclear lymphocytes following the inoculation. Mouse 49A did not develop a tumor. The injected tissue was entirely absorbed September 9th; 19 days after inoculation. The charting of the blood findings of this animal shows a decided increase of the small mononuclear lymphocytes with a corresponding decrease of the polymorphonuclear leucocytes. (See Fig. 6.)

Mouse 49 B was under observation from August fifteenth until October twentieth. On September the fifth, there was a notable fall in the number of polymorphonuclear leucocytes and a rise in the number of small mononuclear lymphocytes. This relationship between the two classes of blood cells continued until September twenty-third, after which there was a constant divergence in the numerical ratio of these cells. The charting of this tumor shows that it diminished in size during this period and that it increased after September twenty-seventh, until it caused the

Fig. 7.



death of the animal October twenty-first. The lines representing the ratio of the small mononuclear lymphocytes and the polymorphonuclear leucocytes diverge and in this respect are similar to those of Exp. II, Figs. 1, 2, 3. (See Fig. 7.)

Experiment II. Ser. 38.

Experiment III. Ser. 49.

Experiment IV. Ser. 72.

[illegible]

Mouse 49 C developed a tumor five days after inoculation. This tumor grew rapidly until on August 30 it weighed about 4.0 gr. The tumor then retrograded spontaneously, and on September 12 it had entirely disappeared. It is of interest to note that the small mononuclear lymphocytes decreased during the period of active tumor growth while the polymorphonuclear leucocytes increased, and that shortly before the retrogression of the tumor became noticeable the relationship between these two classes of cells was entirely changed. (See Fig. 8.)

Fig. 8.

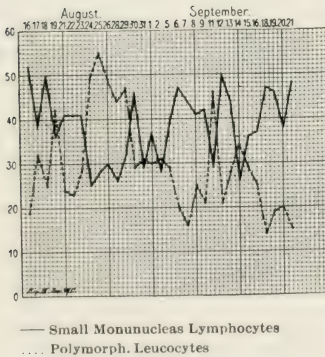
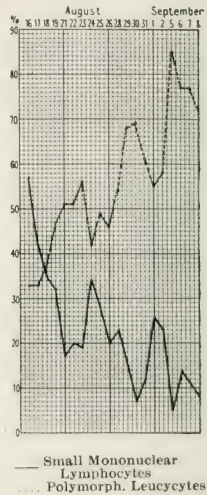


Fig. 9.



Mouse 49 D developed a tumor which grew uninterruptedly until it caused the death of the animal 31 days after inoculation. The chart of the variation of the two classes of blood cells is very much like those of Exp. I, Figs. 1 and 2. (See Fig. 9.)

Experiment IV. Serial No. 72. December 4, 1912.
(See Plate III, p. 19.)

Mouse tumor employed 70 E. Weight of mouse 23.5 gr. Weight of tumor 2.0 gr.

Ratio of tumor to salt solution 1:2. Dose 0.5 c.c.

Cultures made from the tumor, liver and spleen of this mouse were sterile. Five mice were inoculated from this tumor.

Letter	Died	Killed	Weight of		Remarks
			Animal	Tumor	
A ♀ 23.0 gr.	1/3/12	30.0	13.0	9.0 gr. of this tumor had grown by infiltration into the abdominal cavity. This tumor involved the right kidney and the muscles of the back. Culture from tumor sterile.
B ♂ 17.2 gr.	12/30/11	24.0	8.5	The tumor at the root of the tail had broken through 12/26/13. The death of this mouse may have been due to intercurrent infection. Cultures from tumor not sterile.
C ♀ 23.8 gr.	2/3/12	23.0	7.0	Culture from tumor sterile.
D ♀ 22.9 gr.	2/23/12	23.5	5.5	Culture from tumor sterile.
E ♂ 20.0 gr.	3/1/12	30.0	12.5	This mouse drowned in the drinking cup. Cultures from tumor sterile.

In the course of these experiments the question arose whether the variations observed in the differential blood counts of the white cells might not be relative only. To determine this, total blood counts were made about every five days. The blood counts in the animals developing cancer showed an actual decrease in the red cells and an increase of the white cells. Judging from the results of these counts the leucocytosis is actual. The following table gives the blood counts of Exp. IV, Serial No. 72, and may serve as an illustration of the blood findings in the other experiments.

Exp. IV. Serial No. 72.

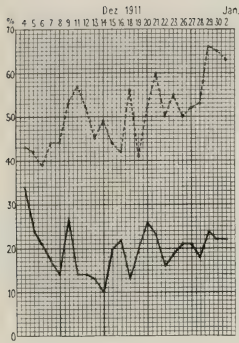
Date	72 A		72 B		72 C		72 D	
	Reds	Whites	Reds	Whites	Reds	Whites	Reds	Whites
12 4/11	10,150,000	12,000	9,600,000	10,000	11,700,000	11,000	10,800,000	9,000
12 9/11	10,380,000	16,000	10,800,000	13,000	11,100,000	10,000	10,280,000	10,000
12 12/11	10,190,000	19,000	9,400,000	19,000	11,500,000	11,000	10,100,000	12,000
12 16/11	8,300,000	17,000	6,100,000	16,000	9,800,000	12,000	10,190,000	16,000
12/21/11	7,600,000	20,000	4,560,000	19,000	7,300,000	17,000	8,100,000	15,000
12 27/11	5,900,000	21,000	4,360,000	22,000	8,600,000	15,000	8,300,000	14,000
12/30/11	4,470,000	23,000	8,400,000	18,000	8,320,000	17,000
1/2/12	7,120,000	19,000	7,600,000	16,000
1/5/12	7,370,000	19,000	6,260,000	17,000
1/11/12	5,940,000	21,000	6,000,000	18,000
1/16/12	8,670,000	22,000	7,500,000	18,000
1/20/12	7,150,000	23,000	7,400,000	20,000
1/25/12	5,290,000	24,000	6,600,000	21,000
1/31/12	4,470,000	25,000	5,390,000	20,000
2/2/12	2,500,000	27,000	4,780,000	22,000
2/7/12	2,300,000	21,000
2/13/12	3,200,000	23,000
2/19/12	3,800,000	23,000

The polymorphonuclear leucocytes and mononuclear lymphocytes showed the following percentage variations on the days complete counts were made on four of the mice of Experiment IV.

Date	Exp IV. 72 A			72 B			72 C			72 D		
	Total No. of white blood cells	% of polymorphonuclear leucocytes	% of small mononuclear lymphocytes	Total No. of white blood cells	% of polymorphonuclear leucocytes	% of small mononuclear lymphocytes	Total No. of white blood cells	% of polymorphonuclear leucocytes	% of small mononuclear lymphocytes	Total of white blood cells	% of polymorphonuclear leucocytes	% of small mononuclear lymphocytes
12/4/11	12,000	43.8	34.9	10,000	66.1	18.9	11,000	38.0	21.9	9,000	40.1	33.1
12/9/11	16,000	55.8	24.4	13,000	59.1	13.3	10,000	53.1	20.9	10,000	54.1	24.6
12/12/11	19,000	52.7	15.2	19,000	67.7	8.3	11,000	61.1	15.8	12,000	50.9	23.1
12/16/11	17,000	42.3	22.2	16,000	52.0	8.2	12,000	46.2	18.0	16,000	57.1	14.7
12/21/11	20,000	60.0	23.0	19,000	58.3	19.6	17,000	35.2	30.0	15,000	40.9	15.3
12/27/11	21,000	52.4	21.8	22,000	61.7	20.7	15,000	44.1	23.5	14,000	55.3	21.3
12/30/11	23,000	45.5	22.7	18,000	42.7	33.4	17,000	42.6	31.3
1/2/12	19,000	41.2	31.3	16,000	49.5	27.5
1/5/12	19,000	44.1	20.1	17,000	45.4	24.8
1/11/12	21,000	50.0	23.7	18,000	40.1	22.1
1/16/12	22,000	61.4	23.4	18,000	64.8	21.9
1/20/12	23,000	57.4	14.4	20,000	57.3	15.6
1/25/12	24,000	61.5	22.6	21,000	45.4	17.5
1/31/12	25,000	64.3	23.7	20,000	67.9	10.5
2/2/12	27,000	68.6	17.9	22,000	61.9	19.5
2/7/12	21,000	61.0	21.5
2/13/12	23,000	63.8	23.9
2/19/12	23,000	72.3	8.5

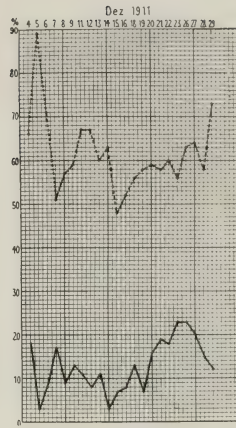
The percentages of large lymphocytes and eosinophiles have been omitted.

Fig. 10.



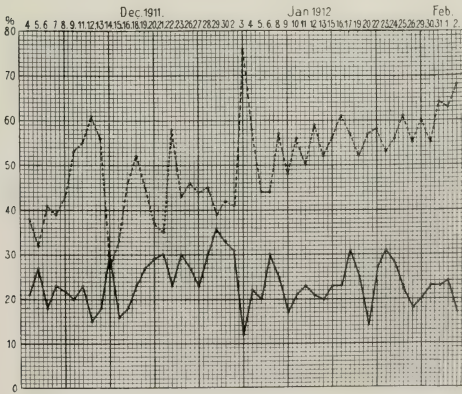
— Small Mononuclear Lymphocytes
 Polymorph. Leucocytes

Fig. 11.



— Small Mononuclear Lymphocytes
 Polymorph. Leucocytes

Fig. 12.



— Small Mononuclear Lymphocytes
 Polymorph. Leucocytes

Fig. 13.

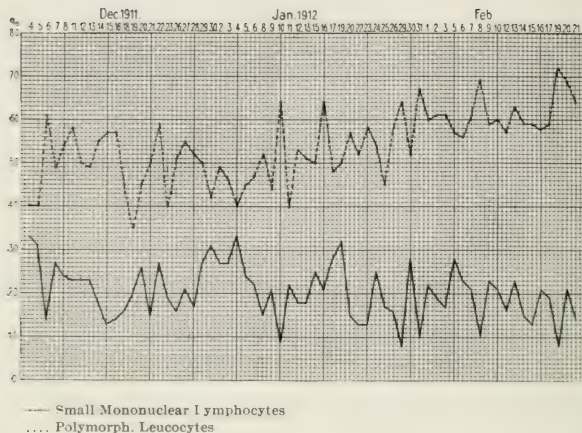
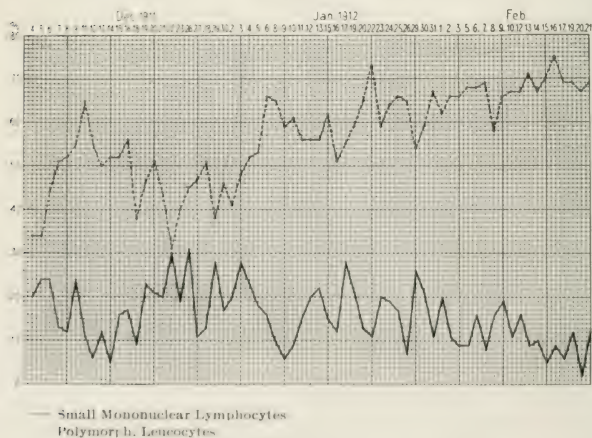


Fig. 14.



The two spontaneous tumor mice which were found in our own stock of breeding mice and included in this communica-

tion presented the same characteristic relationship between the polymorphonuclear leucocytes and the small mononuclear lymphocytes already observed in mice inoculated with transplantable tumor. The two spontaneous tumors were also of the adenocarcinomatous type. (See Figs. 15 and 16.)

Fig. 15.

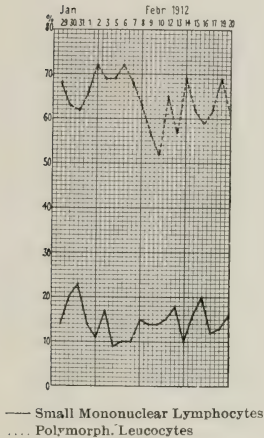
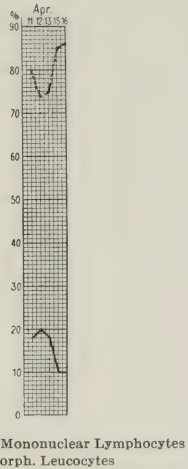


Fig. 16.



ZUSAMMENFASSUNG.

(1) Die Anwesenheit des Carcinoms im Organismus der Mäuse verschiebt das numerische Verhältnis der polynuklearen Leukocyten zu den kleinen mononuklearen Lymphocyten.

(2) Die Leukocytose scheint in Mäusen, die von einem aktiv wachsenden Carcinom befallen sind, konstant zu sein.

(3) Stillstand im Tumorwachstum oder spontane Heilung gehen mit der Verminderung der Leukocytose und der Zunahme der kleinen mononuklearen Lymphocyten Hand in Hand.

(4) Dieselben Verhältnisse finden sich auch in Mäusen, die von spontanem Adenocarcinom befallen sind.

(5) Ob die Lymphocytose und die Rückbildung in einem kausalen Verhältnisse stehen, kann nicht mit Bestimmtheit

angenommen werden, da Lymphocytose oft in rapider Konvaleszenz von solchen Krankheiten beobachtet wird, die durch Verminderung der Lymphocyten gekennzeichnet sind. Die Lymphocytose scheint ein Ausdruck erneuerter physiologischer Aktivität des Körpers zu sein, welche durch die formative Hyperazidität bedingt wird.

REFERENCES.

1. Andrae, Essai d'haematol. pathologie, Paris, 1843, p. 51.
2. Vidal, Arch. général de médecine, Serie 3, T. 8, 1890, p. 333.
3. Lücke, Das Carcinom, Erlangen, 1867.
4. Neubert, Ein Beitrag zur Blutuntersuchung speziell bei der Phthisis pulm. und beim Carcinom, Inaug.-Diss., 1889.
5. Hayem et Alexander (De la leucocytose dans les cancrs.), Compt. rend. de la société etc. 1887, No. 17, Serie 8, T. 4, p. 270.
6. Schneider, Inaug.-Diss. Berlin, 1888.
7. Laache, Langenbeck's Archiv, 1893, p. 486.
8. Cabot, Clinical examination of the blood, 1897, p. 297.
9. Meltzer, S. P., Journ. of A.M.A., June 10, 1911, p. 1753.
10. Gaylord, Clowes and Baeslack, Medical News, Jan. 14, 1905.
11. Da Fano, Fifth Scientific Report on the Investigations of the Imp Cancer Res. Fund, 1912, p. 57.

A STUDY OF THE GERMICIDAL ACTION OF THE ULTRAVIOLET RAYS.

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I. INTRODUCTION.

The possibility of employing ultraviolet light as a sterilizing agent has received much attention within the past few years, particularly in respect to drinking water. It has been found that quantities of water as large as 600 litres an hour have been rendered bacteria-free without difficulty, by exposure to the rays in one of the forms of apparatus now on the market for this purpose^s (Westinghouse-Cooper-Hewitt). The cost and maintenance of the apparatus are, as yet, above the point where the process can enter into active competition with other forms of large-scale water purification, but for smaller installations it has proved successful.

With liquids other than water, such as milk, beer, vinegar, etc., it has thus far been possible to obtain only a partial germicidal action. The applicability of the ultraviolet rays in sterilizing such substances as bacterial vaccines, bouillon, alkaloids, etc., has been studied but little. This investigation was, therefore, undertaken with the view of ascertaining the action of ultraviolet light particularly on these substances.

A résumé of the more important work in this field of sterilization shows that physical considerations, especially the distance of the liquids to be treated from the source of light, also the thickness of the liquid layers, maintain considerable influence. Cernovodeanu and Henri,¹ who were among the earliest workers in this line, found that with bacterial emulsions the germicidal action of the ultraviolet rays decreases more quickly than the reciprocal value of the square of the distance of the liquids from the source of light. They also found the bactericidal action to be much stronger in a layer of liquid 25 cm. in thickness than in one of 2 cm. It did not change with a rise in temperature between

0° and 55°. In general, with water, no chemical or physical changes were observed, except, at most, a small rise of temperature on extended action. Essentially the same conclusions were arrived at in experiments conducted by the Royal German Experiment Station¹⁵ and Courmont, Nogier, and Rochaix²⁰ in their investigation as to the presence of peroxide in water sterilized by ultraviolet light. Henri, Helbronner and de Recklinghausen,⁸ in a study of the sterilization of large quantities of water by the rays, point out the need of allowing sufficient time for the sterilizing action to take place. They suggest for the purpose the use of more than one lamp, and specify that the distance from the lamps to the treated water be as small as possible. Vallet,⁹ in a similar study, calls attention to the necessity of clarifying the water before exposure, and recommends a sterilizing space sufficiently large to permit each particle of water to be exposed to the rays for at least one minute.

The problem of sterilizing milk by ultraviolet rays has been investigated to some extent, particularly by Romer and Sames,⁵ who used a Heraeus mercury-quartz lamp of 6 ampères strength. The milk to be sterilized was contained in a quartz vessel 15 cm. from the source of light, and the rays were allowed to act on a layer of milk 1½ cm. in thickness. The number of bacteria was reduced from 98,900 to 2,050 per cc. after two and one-half hours of action; a second time, they were reduced from 111,800 to 65,000 in twenty minutes' action. With the longer time of action, the taste of the milk, even with incomplete sterilization, became irritating, while, after about an hour's illumination, the experimenters found a distinct destructive action on the oxidases. Butter fat, illuminated for one and three-fourths hours, showed a decrease of 7 per cent. in the iodine number.

An investigation of the action of ultraviolet light on *Bact. tuberculosis* and tuberculin has been made by Cernovodeanu working with Henri and Baroni.¹¹ They placed an equally opalescent emulsion of the bacteria in a quartz tube rotating about the lamp. It was found that the organisms were strongly attacked, even after a short time, being entirely killed after ten minutes of continuous exposure. Tuberculin, exposed to the rays for five minutes or over, gave no reaction when applied to tuberculous guinea pigs.

Stassano and Lematte¹³ in a series of experiments have found that emulsions of various bacteria, after illumination with ultraviolet light, showed the same index to the corresponding agglutinating sera as the living bacilli did. They observed that bacterial life was very quickly destroyed by the rays, but that the substances contained in them, as agglutinins, toxins, enzymes, etc., were not destroyed thereby. They conclude that compared with other methods of sterilization this process has the advantage of not affecting the agglutinins in any manner, and should, therefore, be especially suitable for the preparation of emulsions for sero-diagnostic purposes.

The action of ultraviolet rays on solutions used in pharmacy has been studied to some extent by Lesure.⁶ He found that almost all were as easily penetrable as water, but that the sterilizing action was not the same, using *B. coli communis* as a test organism. Also using the colon bacillus, Vallet⁴ found that dextrose and lactose solutions, glycerine, ethyl alcohol until the appearance of its own bactericidal action, acetic and tartaric acids, were made germ-free when illuminated for one minute in a layer 1.5 mm. in thickness. A 30-per-cent. salt solution, 22-per-cent. soda solution, and 19-per-cent calcium nitrate solution were sterilized under the same conditions. However, the ultraviolet rays were held back and sterilization did not take place when the light had to penetrate a few drops of oil, or when the solution contained 3 per cent. peptone or 1 per cent. albumin.

II. LABORATORY DATA.

A. *Apparatus and Technique Employed.*

In our work with the ultraviolet rays, we used as a source of light a Cooper-Hewitt Standard "Type Y" Quartz Lamp, arranged as shown in Fig. 1 (A). The material to be illuminated was contained in a glass flask (B), connected by a sterile glass syphon and rubber tubing with screw clamps to the trough (C) placed directly beneath the arc. After exposure in the trough, the liquid passed through the delivery tube (D) into a previously sterilized container (E). The arrangement, as used for sterilizing bacterial vaccines, is shown in Fig. 2 with the protective cover in place.

In use, the lamp first caused a heavy drop in voltage with consequent high current which steadily diminished, becoming constant after about six minutes. This is seen from the following electrical data:

Initial voltage (line).....	120 volts
Initial voltage with lamp.....	34 volts
Voltage with lamp (after 6 minutes).....	85 volts
Voltage with lamp (after 15 minutes).....	85 volts
Initial current	8 ampères
Current (after 6 minutes).....	3.5 ampères
Current (after 15 minutes).....	3.5 ampères



Fig. 1. A) Cooper Hewitt Quartz Lamp, used for Sterilizing.

During the operation of the arc, there was a large amount of heat generated which was shown by a rise from 24°C. to 117°C.

in fifteen minutes, the bulb of the thermometer being 6 cm. from the source of light.

As may be partly seen in Fig. 1 (C), the liquids were exposed to the rays in a special, rectangular trough, $15\frac{1}{2}$ cm. x 4 cm., divided into two equal portions by a central septum. The fluid entered at one corner through a tube .5 cm. in diameter and bent at right angles, the trough at this point having a depth of 1.5 cm., but tapering at its other end to 0.8 cm. After flowing through one side, the liquid passed back to the other side by means of a

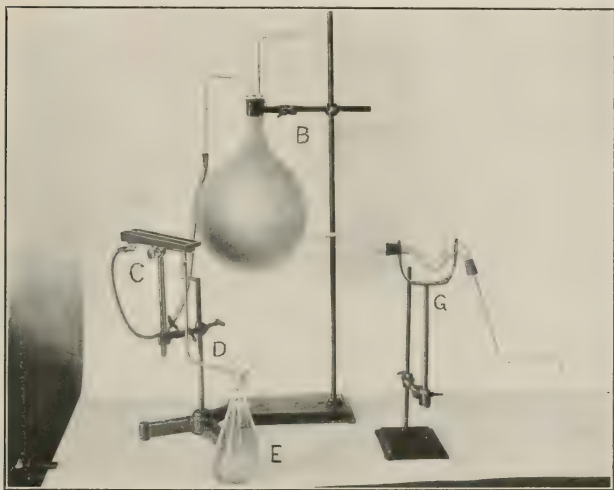


Fig. 1 (B).—Apparatus used with Ultraviolet Light Showing Special Trough and Tube.

diagonal tube 0.7 cm. in diameter, which thus subjected it to a second exposure to the rays. From here, it passed out by means of the bent delivery tube (D) connected to the trough by a union. The whole arrangement was made of brass, heavily nickel-plated, with rounded inside corners, allowing both trough and delivery tube to be sterilized by heating in the free flame. In addition to this the trough was provided in its center with a metal standard and an adjustable screw clamp, thus permitting it to be fixed in any desired position.

In most of the work on water the liquid was exposed to the rays in a special bent quartz tube as shown in Fig. 1 (G), provided with a brass holder and having previously sterilized glass inflow and outflow tubes connected through sterile rubber stoppers. As in the case of the trough, the quartz tube could be sterilized by heating in the free flame. It also permitted a greater rate of flow, but owing to the ease of breakage and difficulties in adjustment, the metal device was found to be much more satisfactory as well as economical.

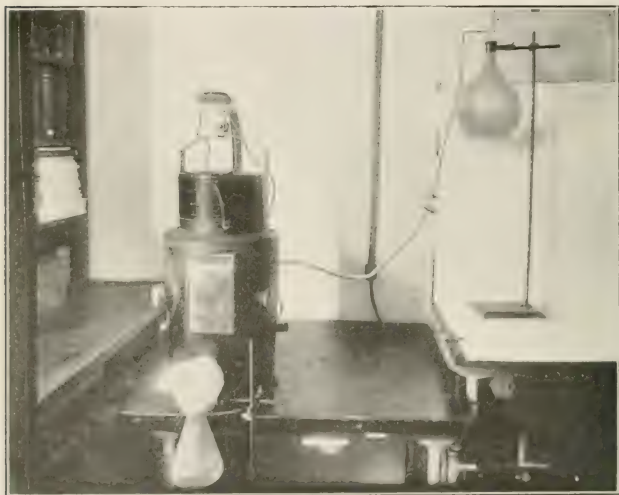


Fig. 2. Complete Apparatus for Sterilization by Ultraviolet Light.

The usual technique consisted in first running through the apparatus about two litres of sterile water into a graduated cylinder. Not only did this serve as a means of regulating the rate of flow, but allowed the lamp to reach the optimum working conditions and, at the same time, cleaned the apparatus. Without breaking the sterile syphon, the material to be tested was now substituted in place of the water, and connected up as shown in Fig. 2. The liquids, after being exposed, were collected under

aseptic conditions in previously sterilized containers, and portions removed to suitable vessels for bacteriological examination.

With the exception of the tap water, the bacterial suspensions and vaccines, *B. coli communis* was used as the test organism for determining the germicidal action of ultraviolet light on the various solutions studied. The inoculation was made in each case by adding to the material an aqueous suspension of the bacillus, shaking well for several minutes, and then estimating the number of bacteria present by plating out 1 cc. samples in various dilutions on suitable culture media.

B. The Action of Ultraviolet Light on Various Substances.

1. *Detroit Tap Water.* Our experiments with the city water supply were carried on during a time when the water was highly turbid, owing to melting ice and heavy winds. There was also a considerable amount of humus material present, giving the water a dark color.

The bacterial content of the water before exposure was obtained by plating out 1 cc. samples in plain agar, and incubating for forty-eight hours at 20°C. After exposure, 5 cc. samples of the treated water were examined. To make sure of the sterility of the media, controls were run at the same time with 5 cc. of sterile water. The results are given in summary in Table I.

The preceding table seems to establish that tap water with considerable matter in suspension and having a bacterial content of 860 organisms per cc., can be sterilized by exposure to ultraviolet rays in a quartz tube at a rate as high as a litre in thirty-eight seconds. Considering the price of electrical energy at 1½ cents per K.W.H., this means that 24 gallons can be sterilized in an hour at about 0.5 cent for cost of power. More data on the comparative cost of water sterilization by ultraviolet rays can be found in the recent studies of M. Recklinghausen,²¹ and I. J. Tanton.²⁸

As would be expected, if the flow of the liquid be slowed down, the heating effects of the lamp are noticeable as in Nos. 11, 12, 13, 21, 22, 23, 42, 43, 44. In fact, where the rate was a litre in forty minutes (Nos. 21, 22, 23), there was a rise of 34°C. in temperature during passage through the apparatus, giving a final temperature of 60°C.—almost sufficient to bring about a decided

TABLE 1.

ACTION OF ULTRAVIOLET LIGHT ON DETROIT TAP WATER.

	Number of Colonies before Exposure in 1 cc.	Flow in Minutes per Litre.	Number of Colonies after Exposure in 5 cc.	Rise in Temper- ature of Water.
1	650	Control	Control	
2	580	"	"	
3	520	"	"	
4	Sterile water		0	
5	As in 1, 2, 3	1.5	0	0
6	" " " "	1.5	0	0
7	" " " "	1.5	0	0
8	" " " "	4	0	0
9	" " " "	4	0	0
10	" " " "	4	0	0
11	" " " "	10	0	4°
12	" " " "	10	0	4°
13	" " " "	10	0	4°
14	500	Control	Control	
15	450	"	"	
16	470	"	"	
17	Sterile water	"	0	
18	As in 14, 15, 16	3.3	0	0°
19	" " " " "	3.3	0	0°
20	" " " " "	3.3	0	0°
21	" " " " "	40	0	34°
22	" " " " "	40	0	34°
23	" " " " "	40	0	34°
24	800	Control	Control	
25	720	"	"	
26	730	"	"	
27	As in 24, 25, 26	0.63	0	0°
28	" " " " "	0.63	0	0°
29	" " " " "	0.63	0	0°
30	" " " " "	0.72	0	0°
31	" " " " "	0.72	0	0°
32	" " " " "	0.72	0	0°
33	" " " " "	1.0	0	0°
34	" " " " "	1.0	0	0°
35	" " " " "	1.0	0	0°
36	" " " " "	2.1	0	0°
37	" " " " "	2.1	0	0°
38	" " " " "	2.1	0	0°
39	" " " " "	4	0	0°
40	" " " " "	4	0	0°
41	" " " " "	4	0	0°
42	" " " " "	14	0	12°
43	" " " " "	14	0	12°
44	" " " " "	14	0	12°
45	Sterile water	Control	0	

germicidal action through heat alone. This fact would be of importance in the case of sterilization of bacterial vaccines.

2. *Aqueous Suspensions of Bacteria.* The presence of *B. coli communis* in 0.1 cc. samples of the tap water used in our experiments brought up the question as to the sterilizing action of the

rays on pure cultures of this organism. Various aqueous suspensions of *B. coli communis*, ranging from 80,000 colonies per cc. to 777,000 per cc., were exposed to ultraviolet light at different rates of flow up to a litre in forty-two seconds. In each case, 5 cc. samples plated out on agar showed no colonies when incubated for three days at 37°C.

To ascertain whether equally efficient results could be obtained with a spore-forming organism, experiments similar to those with the colon bacillus were run with *B. subtilis* alone, and with mixtures of *B. subtilis* and *B. coli communis*, each in numbers of about 200,000 bacteria per cc. Complete sterility was obtained in every case, even with a rate of flow of a litre in thirty-nine seconds.

3. *Beef Bouillon*. The feasibility of sterilizing albuminous liquids by ultraviolet rays was studied with ordinary beef bouillon as used for cultural purposes. To decrease the content of albumins and make the color as light as possible, the bouillon was prepared without the ordinary 2 per cent. peptone, sterilized in streaming steam and then inoculated with *B. coli communis* in the usual manner. The various experiments and results obtained are given in Table II, based on 1 cc. samples on agar, incubated for twenty-four hours at 37°C.

It is easily seen from Table II that the Cooper-Hewitt mercury arc cannot be used for sterilizing bouillon, even with a rate of flow as slow as a litre in ten minutes. Ten minutes for a litre to flow through can be considered the slowest practical rate allowable. Beyond this point the heating effects of the lamp come into consideration.

As may be noted from Nos. 34-39, the percentage reduction (35 per cent. to 60 per cent.) was not so high with more bacteria present as when bouillon containing less bacteria (Nos. 24-29) was exposed to the rays. In the latter case, not only was the reduction somewhat greater (55 per cent. to 65 per cent.) but was also more uniform. The bouillon, itself, also seemed to have a slightly burnt odor after exposure to the ultraviolet light.

4. *Bismarck Brown*. The fact that the bouillon was colored brought up the question as to the effect of color on the action of the rays. To determine this point, water was colored with Bismarck brown till it was equal in intensity to a dark bouillon. It

TABLE II.
ACTION OF ULTRAVIOLET LIGHT ON *B. COLI COMMUNIS* IN BOUILLON
SUSPENSION.

	Number of Colonies before Exposure.	Flow in Minutes per Litre.	Number of Colonies after Exposure.
1	200,000	Control	Control
2	220,000	"	"
3	240,000	"	"
4	As in 1, 2, 3	10	*
5	" " " " "	10	*
6	" " " " "	10	*
7	" " " " "	7	*
8	" " " " "	7	*
9	" " " " "	7	*
10	Control on media		
11	500,000	Control	Control
12	510,000	"	"
13	490,000	"	"
14	As in 11, 12, 13	2.3	*
15	" " " " "	2.3	*
16	" " " " "	2.3	*
17	" " " " "	6	*
18	" " " " "	6	*
19	" " " " "	6	*
20	Control on media		0
21	18,000	Control	Control
22	20,000	"	"
23	21,000	"	"
24	As in 21, 22, 23	2	7,500
25	" " " " "	2	7,200
26	" " " " "	2	7,800
27	" " " " "	1.4	8,500
28	" " " " "	1.4	8,700
29	" " " " "	1.4	8,700
30	Control on media		0
31	750,000	Control	Control
32	730,000	"	"
33	710,000	"	"
34	As in 31, 32, 33	10	320,000
35	" " " " "	10	340,000
36	" " " " "	10	300,000
37	" " " " "	10	440,000
38	" " " " "	10	490,000
39	" " " " "	10	380,000
40	Control on media		0

*Indicates more than 1,000 colonies on plate.

was then infected with *B. coli communis* and run through the apparatus in the usual manner. With rates of flow as high as a litre per minute, complete sterilization took place. Control samples showed that there were about 250,000 organisms per cc. before exposure.

5. *Sodium Chloride.*

6. *Saccharose.* Our experiments with tap water seemed to indicate that dissolved salts did not interfere with the germicidal

action of the mercury arc. This fact was confirmed by exposing to the rays a 1-per-cent., a 2-per-cent., and a 4-per-cent. aqueous solution of common salt, containing as high as 450,000 colon bacilli in every cc. The highest rate of flow was a litre per minute. In every case, 5 cc. samples of the liquid after exposure failed to show any organisms present.

That ionization of the dissolved material has no influence on the sterilization was shown by experiments with saccharose—a typical non-electrolyte. A 4-per-cent. aqueous solution of the sugar was employed, the technique being exactly the same as in the case of the sodium chloride. Here also we found it possible to sterilize with 400,000 *B. coli* per cc. present before exposure.

7. *Urea*. The results obtained with saccharose raised the question as to the effect of the presence of nitrogen on the sterilizing action, since saccharose is a carbohydrate. For this purpose a 1-per-cent. aqueous solution of urea ($\text{CO}(\text{NH}_2)_2$) was infected with *B. coli communis*, the control plates showing 50,000 bacteria per cc. The organisms were found to be entirely killed with rates of flow up to a litre in two minutes. The action at more rapid rates was not studied, but, in all probability, complete sterilization would have been effected.

8. *Glycocoll*. The possible influence on the action of the rays of the simultaneous presence of carboxyl and amino groups in a molecule was studied with a 2-per-cent. aqueous solution of the simplest amino acid—glycocoll ($\text{H}_2\text{N}-\text{CH}_2-\text{CH}_2-\text{COOH}$). In spite of the large number of organisms used in infecting—4,200,000 per cc.—rates of flow of a litre in four, three, and two minutes, respectively, gave no growths when 5 cc. samples of the exposed solutions were plated out on agar at 37°C .

(9. *Leucin*.

(10. *Alanin (alpha)*. Our studies with leucin were undertaken to see whether the length of the molecule as well as its arrangement had any influence on its sterilization by ultraviolet light. Leucin (alpha aminoisocaproic acid) with the formula, $(\text{CH}_3)_2\text{CHCH}_2\text{CH}(\text{NH}_2)\text{COOH}$, is one of the more difficultly soluble higher amino acids. A 1-per-cent. solution of the acid in water was used, and inoculated with the colon bacillus as usual, so that there were about 470,000 bacteria per cc. However, when

exposed to the rays at the rate of a litre in four minutes, it was found impossible to sterilize it.

As may be noted from its formula, leucin has a methyl side chain in its molecule. To make sure that the interference was not due to this methyl group, experiments similar to those with leucin were run with a 1.5-per-cent. solution of Kahlbaum's alpha alanin—the simplest alpha amino acid having a methyl side chain $(\text{CH}_3)_2\text{C}^*\text{H}(\text{NH}_2)\text{COOH}$. The number of organisms present before sterilization was about 2,000,000 per cc., and the maximum rate of flow was a litre in two minutes. In every case the sterilization was found to be complete, showing that the methyl side chain had no influence on the action.

A test of the leucin solution used in our preceding experiments with Millon's reagent gave a distinct red color, showing the presence of proteins. Accordingly, another series of experiments was run with leucin, this time using Kahlbaum's highest purity product. The content of colon bacilli, as shown by control plates, was about 900,000 per cc. with a maximum rate of flow of a litre in two minutes. This time 5 cc. samples of solution after exposure failed to give any colonies on agar, showing that the poor results previously obtained were due entirely to protein compounds admixed with the leucin.

II. *Cocaine Hydrochloride*. The germicidal action of ultra-violet light on alkaloids was studied with 1-per-cent and 2-per-cent. solutions of cocaine hydrochloride in water. One set of solutions was made up with sterile water and with *B. coli* communis as usual, while the other set was made up with clear tap water, to which was added a mold (*Penicillium glaucum*).

In the series where the colon bacillus was used, the number of organisms present ranged from 19,000 per cc. to 160,000 per cc. before exposure to the rays. After the illumination, with a rate of flow of a litre in ten minutes, the number of bacteria remaining was from 200 in every cc. to several thousand per cc. With more rapid rates of flow, the number of organisms remaining ranged much higher.

Even at the slowest rates of flow, the solutions infected with the mold showed apparently no reduction in colonies after exposure to the rays. From the results obtained with cocaine, it would seem that the alkaloids, probably on account of their high

molecular weight, do not permit of sterilization by the ultraviolet rays. Comparative tests of its anæsthetic properties on dogs before and after exposure to the ultraviolet light showed that the cocaine hydrochloride was not materially affected. Both with the bacteria and mold, the action seemed to be the same in the 1-per-cent. as in the 2-per-cent. solutions.

12. *Suspensions of Mold in Water.* The poor results obtained with the cocaine solutions containing mold brought up the question as to the action of ultraviolet rays on mold. For this purpose, aqueous suspensions of different species of *Penicillium*, *Aspergillus* and *Mucor* were prepared, so that the number of colonies, when properly diluted and sown on alkaline agar, ranged from about fifty to several hundred per cc. When exposed to the rays at a rate of flow as slow as a litre in twelve minutes only a small destructive action was obtained. The percentage reduction was greater where there were more fungi present before exposure, but in no case did it exceed 20 per cent. The data obtained seemed to show conclusively that mold cannot be killed by ultraviolet light, except when present in very small amounts, as one or two colonies per cc.

13. *Milk.* In our studies on the sterilization of milk by ultraviolet light, we used a Jersey milk, rich in butter fat, and about twenty hours old. The laboratory results are given in Table III. Both plain (P. A.) and litmus-lactose agar (L. L. A.) were used as culture media. One cc. samples were plated out and incubated for forty-eight hours at 37°C. The red colonies, as seen on the litmus-lactose agar plates, have been designated by (r), and are given together with the total number of colonies on the plate.

Confirming the results of Romer and Sames,⁵ and those more recent of Huyge,¹⁸ Ayers and Johnson,³² Table III shows that the ultraviolet rays cannot be successfully used for sterilizing milk. This would have been expected from our results with bouillon. Huyge believes that the difficulty in the sterilization of milk is due to the colloids present.

At the slowest possible rate of flow—a litre in ten minutes—the greatest reduction in bacterial numbers was about 45 per cent. (No. 11). Where the organisms were not so numerous (Nos. 14-21), a still greater reduction was obtained—the highest being almost 70 per cent. in No. 20. Macroscopical and microscopical

TABLE III.

THE GERMICIDAL ACTION OF ULTRAVIOLET LIGHT ON MILK.

	Number of Colonies before Exposure in 1 cc., P. A.	Number of Colonies before Exposure in 1 cc., L. L. A. X 1,000	Rate of Flow in Minutes per Litre.	Number of Colonies after Exposure in 1 cc., P. A.	Number of Colonies after Exposure in 1 cc., L. L. A. X 1,000.
1	204,000	75(37r)	Control	Control	Control
2	189,000	53(28r)	"	"	"
3	196,000	52(33r)	"	"	"
4	As in 1, 2, 3	As in 1, 2, 3	2	130,000	84(49r)
5	" " " " "	" " " " "	2	140,000	80(52r)
6	" " " " "	" " " " "	2	143,000	79(46r)
7	" " " " "	" " " " "	5	120,000	65(40r)
8	" " " " "	" " " " "	5	126,000	69(38r)
9	" " " " "	" " " " "	5	119,000	62(36r)
10	" " " " "	" " " " "	10	115,000	54(32r)
11	" " " " "	" " " " "	10	109,000	67(46r)
12	" " " " "	" " " " "	10	110,000	50(32r)
13	Control on Media				
14	26,000	8.4(6.1r)	Control	Control	Control
15	24,000	9.2(6.0r)	"	"	"
16	30,000	11.0(7.2r)	"	"	"
17	As in 14, 15, 16	As in 14, 15, 16	7	10,000	4.9(3.1r)
18	" " " " "	" " " " "	7	9,500	4.6(2.9r)
19	" " " " "	" " " " "	7	9,200	4.8(3.2r)
20	" " " " "	" " " " "	7	9,100	4.0(3.2r)
21	" " " " "	" " " " "	7	9,800	4.2(2.7r)

examination of the colonies on the litmus-lactose agar plates, both before and after exposure to the rays, showed a preponderance of the *Bact. lactis acidi* group after exposure. A curious fact which can be seen from the table (Nos. 4-12) is that there were more colonies growing on the litmus lactose agar plates after exposure than before.

It would seem, then, that exposure of milk to ultraviolet rays has a tendency to kill off the undesirable organisms present, leaving the more desirable bacteria of the *Bact. lactis acidi* group in the majority. There was no perceptible change in the taste of the milk after being illuminated.

14. *Wine*. Contrary to what might be supposed from the preceding work, it was found possible to completely sterilize wines by the ultraviolet rays. The wines used in our experiments

were a Tokay and a Sherry, each containing about 16 per cent. alcohol, and both colored. On account of the relatively high percentage of alcohol, the infecting organism in this case was a spore-forming bacillus of the *B. subtilis* group, already present in the Sherry wine when obtained. This bacillus was isolated in pure culture and was also used for infecting the Tokay wine.

The number of infecting organisms ranged from about 25,000 to several million per cc., as shown by plating out on dextrose agar at 37°C. The best rate of flow seemed to be about a litre in five minutes. Slower rates imparted a sort of burnt taste to the wines, while higher rates did not permit of complete sterilization. Confirming our results with Bismarck brown, the dark color of the Sherry wine did not seem to interfere with the germicidal action of the rays.

15. *Adrenalin Chloride Solution.* Our work with Adrenalin Chloride was mostly with a 1:3200 aqueous solution preserved with chloretone. Several experiments with solutions in which the chloretone had been removed seemed to indicate that sterilization was possible when the liquid was exposed to the rays at the rate of a litre in six minutes. *B. coli communis* was used as the test organism.

However, after exposure to the rays the adrenalin solutions showed a distinct red coloration, indicative of deterioration. Physiological assays by injection into the circulatory system of dogs, both before and after the illumination, showed that after exposure there is a loss of 30 per cent. in the physiological activity. The action is probably one of oxidation. More work with this substance is contemplated.

16. *Bacterial Vaccines.* Our previous work showed that ultraviolet light could be used to sterilize salt solutions. This suggested the use of the rays in the preparation of bacterial vaccines. As would be expected, the rate at which the vaccine is exposed to the rays is of the greatest importance, owing to the large number of organisms present. Where the number was fifty million or more per cc. the minimum rate at which we found complete germicidal action to take place was a litre in five minutes. With streptococci and the micrococci (staphylococci), slower rates were necessary to kill all of the organisms. A rate

of flow of a litre in eight minutes seemed to be the most satisfactory for all of the vaccines studied.

In preparing the vaccines, the organisms were grown as usual on the appropriate culture media for the necessary length of time. The growth was then washed off with sterile physiologic salt solution, the bacterial numbers estimated by Wright's method, and the suspension diluted with sterile salt solution to give the desired number of organisms per cubic centimeter. After shaking for at least half an hour, in a shaking machine, the diluted suspension was exposed to the ultraviolet rays, and run directly into a sterile container under aseptic conditions. The preliminary shaking was found to be absolutely necessary with the streptococci, probably due to clumping of the organisms and thus escaping the action of the rays.

THE ACTION OF ULTRAVIOLET LIGHT ON VARIOUS SUBSTANCES

Substance.	Number of Organisms per cc. before Exposure.	Rate of Exposure to Rays Minimum per Litre.	Number of Colonies per cc. after Exposure.	Changes Produced.
1. <i>Detritus tap water.</i> (Highly turbid).	400-800	38 sec.	Sterilized	None
2. <i>Aq. suspensions.</i> <i>B. coli communis</i> <i>B. subtilis</i>	80,000-777,000	42 sec.	Sterilized	None
	170,000-400,000	39 sec.	Sterilized	None
	400,000	39 sec.	Sterilized	None
3. <i>Beti bouillon.</i> <i>B. sub. & B. coli.</i> <i>B. coli com.</i>	18,000-750,000	1 4-10 Min.	7,500-490,000	Slight burnt odor
4. <i>Bismarck brown sol.</i> <i>B. coli comm.</i>	250,000	1 Min.	Sterilized	None
5. <i>Sodium chloride, 1%.</i> 2% 4% aq. sol.	48,000-450,000	1 Min.	Sterilized	None
6. <i>Saccharose 4% aq. sol.</i> <i>B. coli comm.</i>	400,000	1 Min.	Sterilized	None
7. <i>Urea. 1% aq. sol.</i> <i>B. coli comm.</i>	10,000-50,000	2 Min.	Sterilized	None
8. <i>Glycerol 2% aq. sol.</i> <i>B. coli comm.</i>	670,000-4,200,000	2 Min.	Sterilized	None
9. <i>Leucine impure 1% aq. sol.</i> <i>B. coli comm.</i>	470,000	4 Min.	Unsterilized	None
	900,000	2 Min.	Sterilized	None
10. <i>Leucine (Kahlbaum's Best) 1% aq. sol.</i> <i>Alkali adipic 1.5% aq. sol.</i>	2,000,000	2 Min.	Sterilized	None
11. <i>Glycerine hydrochloride.</i> 1% and 2% aq. sol.	19,000-160,000	10 Min.	200-2700 <i>B. coli comm.</i> 180 mold.	None
12. <i>Suspension of mold in water.</i>	200 mold.	12 Min.	44-220	None
13. <i>Milk (Jersey).</i> (48 hour, 37° count)	24,000-240,000	7-10 Min.	9,100-143,000	None
14. <i>Waxes (Sheep and Tokay)</i> (25,000-2,900,000 (<i>B. sub. group</i>))	25,000-2,900,000	5 Min.	Sterilized	None. Burnt taste with rates faster than 5 min.
15. <i>Adrenalin chloride sol. sol.</i> (1:32000)	60,000 <i>B. coli comm.</i>	6 Min.	Sterilized	Red Coloration 30% loss in physiologi- cal activity.
16. <i>Reverted vaccines.</i> a) <i>Colon.</i> b) <i>Colon typ.</i> c) <i>Colon (comb.)</i> d) <i>Strept. (")</i> e) <i>Staph. (")</i> f) <i>Gonorr. (")</i>	a) 50,000,000 b) 100,000,000 c) 400,000,000 d) 50,000,000 e) 500,000,000 f) 900,000,000	a) 5 Min. b) 7 " " c) 8 " " d) 10 " " e) 10 " " f) 10 " "	a) Steril. b) " " c) " " d) " " e) " " f) " "	None " " " " " " " " " "

We have prepared in this way the following vaccines :

- | | | | | | | |
|---|--------------------------|------------------|------------------|---|---|---|
| 1 | Colon vaccine— | 50,000,000 | bacteria per cc. | | | |
| 2 | Colon-typhoid vaccine | —100,000,000 | bacteria per cc. | | | |
| 3 | Colon vaccine (combined) | —400,000,000 | | “ | “ | “ |
| 4 | Streptococcus vaccine | “ — 50,000,000 | | “ | “ | “ |
| 5 | Staphylococcus | “ “ —500,000,000 | | “ | “ | “ |
| 6 | Gonorrheal | “ “ —900,000,000 | | “ | “ | “ |

Sterility was shown in each case by the absence of any growth when 5 cc. samples were sown in plain and dextrose agar at 37°C. Owing to the high turbidity and the very great number of organisms present, it was found necessary to use a rate of flow of a litre in ten minutes with the gonorrheal vaccine. Ascitic agar was used here to determine the sterility. All of the vaccines were preserved in the regular manner by the addition of 0.2-per-cent. trikresol.

It is thought by some investigators that the usual method of preparing vaccines by heat impairs the keeping qualities and the power of producing antibodies. (Cf. Semple and Matson (27)). On the other hand, the excess of chemicals which might be required to effect sterilization may produce irritation. Theoretically, then, vaccines prepared by ultraviolet light should possess superior therapeutic action. A clinical study, using several vaccines so prepared, is being made with a view of determining this point. This, together with more researches on ultraviolet light sterilization, we hope to report upon in a future publication.

III. DISCUSSION.

Several theories have been advanced as to the mode of action of the ultraviolet rays. The hypothesis advanced by Glaser¹⁴ seems to be plausible. He explains it on the photo-mechanical theory that by the continued action of ether vibrations on the organisms, the number of vibrations is brought to a point of rupturing the chemical combinations.

We have noticed, in our own work, the formation of ozone during the burning of the mercury arc. This can be detected by the odor, or by the fact that strips of moistened potassium-

iodide starch paper placed in the vicinity of the rays are turned blue. The theory has been advanced that the germicidal action of the rays is due to the formation of ozone or hydrogen peroxide. Oker-Blom,²⁵ from recent ultraviolet light studies with a Nogier-Triquet mercury arc, concludes that the germicidal effects have no basis as being due to the action of nitrous acid, ozone or hydrogen peroxide. It must rather be considered as due to the direct action of short-wave rays on the living bacterial protoplasm. It may be possible that the action can also influence secondary chemical reactions taking place through the agency of ultraviolet light.

The fact that secondary chemical changes may be produced by the rays was seen from the change we found in the adrenalin chloride solutions after the exposure. Berthelot and Gaudechon²³ have found that the rays are able to decompose solutions of levulose, while acetone is partly changed to acetic acid. Massol¹⁹ has found that under the action of ultraviolet, starch gradually loses its tinting power with iodine. More recently Euler and Ryd²² have found that lactic acid undergoes, through the agency of ultraviolet rays, a splitting up which may be considered analogous to the fermentative cleavage of this acid. Similarly, carbon dioxide is split off from tartaric acid by the action of the rays, a reducing substance being formed at the same time.

As noted, particularly with the bacterial vaccines, turbidity rapidly decreases the germicidal action of the rays. However, as Oker-Blom²⁴ has also found with water, if the turbidity be not too high, it is still possible to sterilize with decreased rates of flow. Our work with vaccines also showed that bacteria have a varying resistivity to the action of the rays. The streptococci and staphylococci were the most difficult to kill, while the spore-forming types offered no apparent resistance. Confirming our own results, Oker-Blom finds that color due to vesuvine does **not** materially affect the sterilization.

Considerable discussion has arisen of late in regard to the most efficient type of lamp from the germicidal point of view. Two general types of mercury arc sterilizers can be distinguished, viz.:

1. The "over-water" type or "non immersed" type, where the arc is above the material to be acted upon. The Westinghouse-

Cooper-Hewitt lamps, similar to the one used in our own experiments, are of this kind.

2. The "under-water" or "immersed" type, where the arc is immersed in the fluid to be illuminated. The Courmont-Nogier and Nogier-Triquet lamps are of this type.

Theoretically, the immersed type should be more efficient, owing to all of the rays being utilized, and the elimination of the intervening air space. On the other hand, the "over-water" type is more adaptable for acting on materials other than water. By burning at a higher temperature, it also produces a greater amount of the shorter or germicidal rays. For large-scale water sterilization, it would seem that the Nogier-Triquet type is more suitable, while for general, small-scale sterilization, the Cooper-Hewitt (non-immersed) type seems to be more efficient. An able discussion of this point is to be found in a recent article by Nogier.²⁷

IV. CONCLUSIONS.

A. The ultraviolet rays produced by the Cooper-Hewitt mercury arc have a strong bactericidal action.

B. Certain species of bacteria in aqueous suspension, including spore-forming organisms, are killed by exposure to the rays. Molds, however, are only partially destroyed by the ultraviolet light.

C. The action seems to be a photo-mechanical process, and is, in all probability, due to absorption of the ultraviolet rays by the bacterial protoplasm.

D. Water, wines, many inorganic and a number of organic substances in aqueous solution, can be sterilized by ultraviolet light. Bacterial vaccines require a prolonged action.

E. Proteins and other bodies of high molecular weight interfere with the action of the rays. Turbidity, both organic and inorganic, has a similar action. Color, within certain limits, seems to have no influence.

BIBLIOGRAPHY.

1. *Cernovodeanu and Henri*, Compt. rend. de l'Acad. des sciences, 1910, V. 150 p. 52.
2. *Billon and Daguerre*, Compt. rend. de l'Acad. des sciences, 1910, V. 150, p. 479.
3. *Urbain, Scal and Feige*, Compt. rend. de l'Acad. des sciences, 1910, V. 150 p. 548.
4. *Vallet*, Compt. rend. de l'Acad. des sciences, 1910, V. 150, p. 632.
5. *Romer and Sames*, Hyg. Rundschau, 1910, No. 16.
6. *Lesure, Jr.* de Pharm. et de Chimie, 1910, Ser. 7, pt. 1, p. 569.
7. *Cernovodeanu and Henri*, Compt. rend. de l'Acad. des sciences, 1910, V. 150, p. 729.
8. *Henri, Helbronner and de Recklinghausen*, Compt. rend. de l'Acad. des sciences, 1910, V. 150, p. 932.
9. *Vallet*, Compt. rend. de l'Acad. des sciences, 1910, V. 150, p. 1076.
10. *Courmont, Nogier and Rochaix*, Compt. rend. de l'Acad. des sciences, 1910, V. 150, p. 1453.
11. *Baroni, Cernovodeanu and Henri*, Compt. rend. de l'Acad. des sciences, 1910, V. 151, p. 724.
12. *v. Recklinghausen*, Gesundheits Ingenieur, 1911, V. 34, p. 116.
13. *Stassano and Lematte*, Compt. rend. de l'Acad. des sciences, 1911, V. 152, p. 623.
14. *Glaser*, Wiener klin. Wochenschr, 1911, No. 32, p. 1150.
15. Mitteilung aus der Kgl. Prüfungsanstalt für Wasserversorgung, 1911, V. 1, p. 85-102.
16. *Borinski*, Gesundheits Ingenieur, 1912, V. 35, p. 779.
17. *Clemesha*, Ind. Med. Gazette, 1912, p. 267.
18. *Huyge*, Annuaire de la Stat. agronom. de l'Etat a Gembloux, 1912,—Ministere de l'Agriculture et des Travaux publics Bruxelles.
19. *Massol*, Compt. rend. de l'Acad. des sciences, 1912, V. 154, p. 1564.
20. *Henri*, Comp. rend. soc. biol., 1912; a. V. 73, No. 26, p. 323; b. V. 73, No. 28, p. 319; c. V. 73, No. 28, p. 321; d. V. 73, No. 28, p. 326; e. V. 73, No. 28, p. 328.
21. *Recklinghausen*, Rev. d'Hyg. et de Police sanit., 1912, V. 34, No. 12, p. 1449.
22. *Euler and Ryd*, Biochemische Zeitschrift, 1913, V. 51, p. 97.
23. *Berthelot and Gaudechon*, Compt. rend. de l'Acad. des sciences, 1913, V. 156, No. 9, p. 707.
24. *Oker-Blom*, Zeitschr. f. Hyg. u. Infektskr., 1913, V. 74, pt. 2.
25. *Oker-Blom*, Zeitschr. f. Hyg. u. Infektskr., 1913, V. 74, pt. 2, p. 242.
26. *La Presse Medicale*, 1913, No. 32 (April 19).
27. *Nogier*, La Presse Medicale, 1913, No. 43, p. 613.
28. *Tanton*, Rev. d'Hyg. et de Police sanit., 1913, V. 35, No. 1, p. 1.
29. *Marinier*, Rev. d'Hyg. et de Police sanit., 1913, V. 35, No. 1, p. 24.
30. *Moycho*, Compt. rend. de l'Acad. des sciences, 1913, V. 156, No. 16, p. 1268.
31. *Henri and Bielecki*, Compt. rend. de l'Acad. des sciences, 1913, V. 156, No. 17, p. 1322.
32. *Ayres and Johnson*, Journ. of Washington Acad. of Sciences, 1913, V. 3, p. 160.

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32. Studies on the Virus of Hog Cholera. By Walter E. King and F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 39-41.)
33. The Physiological Activity of *Cannabis Sativa*. By H. C. Hamilton, A. W. Lescobier and R. A. Perkins. (*Journal of the American Pharmaceutical Association*, Vol. 2, Jan., 1913, pp. 22-30.)
34. The Iodine Content of the Small, Medium and Large Thyroid Glands of Sheep, Beef and Hogs. By T. B. Aldrich. (Original Communications, Eighth International Congress of Applied Chemistry, Vol. XIX, 1912, pp. 9-14.)

35. Studies on the Virus of Hog Cholera. By Walter E. King and Robert H. Wilson. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 16, Heft 3, 1913, pp. 367-376.)

36. On the Cultivation of the Treponema Pallidum (Spirochæta Pallida). By F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 55-67.)

37. Studies on the Gonococcus. I. By Carl C. Warden. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 93-105.)

38. Studies on the Virus of Hog Cholera. By Walter E. King, F. W. Baeslack and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 12, March, 1913, pp. 202-205.)

39. Bacillus Bronchisepticus—Its Relation to Canine Distemper. By N. S. Ferry. (*American Veterinary Review*, Vol. 43, April, 1913, pp. 16-30.)

40. Drug Influence on Extrasystoles of the Mammalian Heart. By Carey P. McCord. (*Interstate Medical Journal*, Vol. 19, Oct., 1912, pp. 870-880.)

41. The Employment of Protective Enzymes of the Blood as a Means of Extracorporeal Diagnosis. I.—Sero-Diagnosis of Pregnancy. By Carey P. McCord. (*Surgery, Gynecology and Obstetrics*, Vol. 16, April, 1913, pp. 418-421.)

42. Tribromo-tert-Butyl Alcohol, $C_4H_7OBr_3$. By T. B. Aldrich. (*Journal of the American Chemical Society*, Vol. 33, March, 1911, pp. 386-388.)

43. On Feeding Young White Rats the Posterior and the Anterior Parts of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 31, Nov., 1912, pp. 94-101.)

44. The Rationale of the Use of Adrenalin in the Treatment of Asthma. By Carey P. McCord. (*Medical Record*, Vol. 83, March 8, 1913, pp. 431-432.)

45. Standardization of Disinfectants: Some Suggested Modifications. By H. C. Hamilton and T. Ohno. (*American Journal of Public Health*, Vol. 3, June, 1913, pp. 582-588.)

46. Preventive Measures Against Equine Influenza Based on Its Bacteriology. By N. S. Ferry. (Report of the Proceedings of the United States Live Stock Association, December, 1912, p. 127.)

47. Correcting Water. By H. C. Hamilton. (*Bulletin of Pharmacy*, Vol. 27, August, 1913, pp. 330-335.)

48. Duration of Immunity Following Small-pox Vaccination. By A. W. Lescohier. (*Journal of the American Medical Association*, Vol. 66, Aug. 16, 1913, page 487-490.)

49. On Crystalline Kombe-Strophanthin. By D. H. Brauns and O. E. Closson. (*Journal of the American Pharmaceutical Association*, May, June and July, 1913, Vol. 2.)

50. A Comparative Study of Antigens for the Wassermann Reaction. By H. R. Varney and F. W. Baeslack. (*Journal of the American Medical Association*, Vol. 66, Sept. 6, 1913, pp. 754-757.)

51. The Treatment of Tetanus. By Charles T. McClintock and Willard H. Hutchings. (*Journal of Infectious Diseases*, Vol. 13, Sept., 1913, pp. 309-320.)

52. Spirochæta Suis, Its Significance as a Pathogenic Organism, Studies on Hog Chlorea. By Walter E. King and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 13, Nov., 1913, pp. 463-498.)

53. Time Recorder for Kymograph Tracings. By Oliver E. Closson. (*Journal of Pharmacology and Experimental Medicine*, Vol. 5. Jan., 1914. pp. 235-238.)

54. U. S. P. Menstrua. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 86, Feb., 1914, pp. 56-61.)

55. Numerical Variations of the White Blood Cells in Mice Inoculated with Transplantable Adenocarcinoma. By F. W. Baeslack. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 20, Heft 5, 1914, pp. 421-435.)

56. A Study of the Germicidal Action of the Ultraviolet Rays. By E. M. Houghton and I. Davis. (*American Journal of Public Health*, Vol. 4, March, 1914, pp. 224-240.)

SOME PHENOMENA INVOLVED IN THE LIFE HISTORY OF SPIROCHAETA SUIIS.*†

STUDIES ON HOG CHOLERA.

WALTER E. KING AND RAYMOND H. DRAKE.

(From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.)

The recognition of *Spirochæta suis*¹ in certain lesions found in cases of hog cholera, and the results attending experimental cultivation and animal inoculation have afforded many interesting observations relative to the life history of the organism. These studies have not yet progressed to such a point that definite conclusions can be drawn as to the cycle through which this organism may pass. However, its morphological variations under different conditions appear to correspond in many respects with those of other spirochetes which have been studied and reported upon by many investigators.²

A future publication, which we hope will contribute toward the interpretation of the life cycle of spirochetes in general, will contain more detailed results of our observations of *Spirochæta suis*, and its morphological and biological variations. This preliminary report is submitted in order that the results of certain filtration experiments may be placed on record.

Dujardin-Beaumetz³ in discussing *Die Peripneumonie der Rinder* refers to certain interesting filtration experiments as follows:

*Received for publication January 19, 1914.

†Owing to the provisional use of the name "*Spirochæta suis*" by Bosanquet (*Spirochetes*, Saunders, 1911) and Neveu-Lemaire (*Parasitologie des Animaux Domestiques*, Paris, 1912) in referring to the findings of Dodd (*Jour. Comp. Path.*, 1906, 19, p. 216) and Cleland (*Parasitology*, 1908, 1, p. 218), it will be necessary to designate this organism by another name. This will be done in future publications.

¹King and Baeslack, *Jour. Infect. Dis.*, 1913, 12, p. 307; King, Baeslack, and Hoffmann, *Jour. Infect. Dis.*, 1913, 12, p. 365; King and Hoffmann, *Jour. Infect. Dis.*, 1913, 13, p. 463.

²Gleitsmann, *Centralbl. f. Bakteriöl.*, I, Orig., 1913, 68, p. 31; Mühlens, *Handbuch d. Path. Protozoen*, Prowazek, Leipzig, 1912, p. 361; Ross, *Brit. Med. Jour.*, 1912, p. 1651; McDonagh, *Lancet*, *Proc. Roy. Soc.*, 1913, 6, p. 86; Dutton, *Jour. Trop. Med.*, 1907, 10, p. 385; Mayer, *Arch. f. Schiffs- u. Trokenhyg.*, 1908, 12, Beihefte 1, p. 1; Mackinnon, *Parasitology*, 1909, 2, p. 267; Bosanquet, *Spirochetes*, Saunders, 1911; Marchoux and Couvy, *Ann. de l'Inst. Past.*, 1913, 27, p. 450; Meirowski, *München. med. Wchnschr.*, 1913, 60, pp. 1870 and 2042. Fry, Ranken, and Plimmer, *Jour. Royal Army Med. Corps*, 1913, 21, p. 137; Balfour, *Centralbl. f. Bakteriöl.*, I, Orig., 1913, 70, p. 182; O'Farrell and Balfour, *Centralbl. f. Bakteriöl.*, I, Ref., 1913, 59, p. 292; Noguchi, *Jour. Exper. Med.*, 1912, 16, p. 199.

³*Handbuch d. Path. Mikroog.*, 1913, 8, p. 943.

"ISOLATION OF BACTERIA BY FILTRATION."

"In investigations of pleuropneumonia, various writers have often filtered the pleuropneumonia exudate through 'Gips' and porcelain filters and observed that such filtrate is incapable of causing either infection or immunity in the animals. From this fact it was learned that the pleuropneumonia was not caused by a soluble virus, but by a living organism. But when Löffler had proved that it was possible to infect animals with lymph obtained from aphthous pustules, diluted with water in proportion 1:50 and then filtered, and that there were therefore bacteria which were so infinitely small that they would pass through the filter, the investigations on pleuropneumonia were again taken up, along the lines where positive results were obtained by means of the dilution method. The method of dilution, as well as the quality of the filter, is of the greatest importance for the success of the experiment. If pleuropneumonia exudate or a culture in bouillon, which contains more than 15 per cent of serum, is used for filtration without being previously diluted, failure is usually the result, since the pores of the filter become clogged with the albumen. The choice of candle is not without significance as the different filters possess different degrees of porosity. . . . The passage of this organism through certain filtering walls is of the greatest interest from the standpoint of bacteriologic technic. The filter, which until now has been regarded as a perfect process for the sterilization of warmed fluid media, can also be of great service in the isolation of very small bacteria, by retaining the other larger microbes in their walls. It might also be possible to isolate and compare the filtrable viruses serially, using for this a set of suitably chosen filters of decreasing porosity.

"If the possibility is also offered of obtaining the pleuropneumonia virus in pure state, it is always wise to inoculate cultures in serum bouillon. If the presence of foreign bacteria in the inoculation material is feared, a preliminary culture on serum agar can be made; this, however, is not advisable. Pleuropneumonia develops very slowly on solid media, and the colonies are not visible until after 3 days' standing at 37°. During this time the other more rapidly developing bacteria, such as the pleuropneumonia bacillus of Arloing, overgrow the surface of the agar, and render isolation of the pleuropneumonia organism impossible. By employment of the filtration method this obstacle is overcome. The fluid can be collected without any special precautions. It can be taken even in the stage of decomposition and still be capable, by filtration, of isolating the organism in pure state. The process is as follows: The lung lymph is diluted in proportion of 1:100 with peptone bouillon containing no serum. The bouillon must be filtered through a porcelain filter, not for purpose of sterilization, but to eliminate all solid particles which are suspended in the fluid and could hinder filtration. The lymph diluted in the above manner is then filtered through Berkefeld or Chamberland filters L. It is recommended that the bouillon be first warmed at 37-39°, for it is known that warm fluids can pass through porous strata better, and the passage of the virus is thereby guaranteed. For the certainty of

the growth of the specific cause, serum must be added to the filtrate so obtained, in the proportion of 10 parts serum to 100 parts bouillon. To prevent accidental contamination, it is well to filter the serum through the same filter as is used for the filtration of the bouillon. The filtrate, mixed with serum, is placed in the incubator, and after 3-4 days an opalescence is observed in the cultures. If such an opalescence also appears in those tubes which were further inoculated with material from these cultures, and if no such change can be observed in the control tubes which were inoculated at the same time, the growth of the pleuropneumonia virus is proved, and the costly animal experiment is unnecessary. It is sufficient if only a trace of the opalizing fluid is spread on the serum agar. Very fine colonies, at first transparent, later wartlike, appear on this medium in so characteristic a form as to banish any doubt as to their identity.

"This method can be used not only for the purpose of isolation, but also for veterinary diagnosis in the investigation of lung material obtained from cattle which have succumbed to suspected pneumonia."

In a former publication¹ data were presented showing that the Berkefeld filtrates of cultures containing *Spirochæta suis* were capable of producing hog cholera in susceptible hogs. In the meantime successful attempts have been made to develop cultures of the spirochete from the Berkefeld filtrates. This work, of which brief notes are given, was undertaken chiefly for the purpose of securing data bearing on three important points: (1) to determine the correlation between the "filtrable virus," or "ultramicroscopic organism" of hog cholera, and *Spirochæta suis*; (2) to secure pure cultures of the spirochete; and (3) to determine the importance of the granules assumed to be related to *Spirochæta suis*.

September 23, 1913: A small portion of necrotic tissue containing numerous *Spirochætæ suis*, from ear of Hog 653, was shaken in sterile water and filtered through Berkefeld filter N, pressure less than one atmosphere, time of filtration about 20 mins., and volume of filtrate about 10 c.c. Cultures from the filtrate were made in broth, grown aerobically, and found to be sterile.

Special media, inoculated with the filtrate and incubated under anaerobic conditions, were placed in a desiccator, *in vacuo*, with pyrogallie acid. The media consisted of (1) Hata medium,² with hog blood corpuscles, and (2) Hata medium with rabbit kidney tissue and 0.5 per cent phenol.

¹*Jour. Infect. Dis.*, 1913, 13, p. 484.

²Hata, paper read at Seventeenth International Congress of Medical Sciences, London, August, 1913.

DARK-FIELD EXAMINATIONS.

October 18, 1913: One culture of each medium showed many granules but no spirochetes.

October 31, 1913: One culture of each showed slight contamination with a bacillus, no spirochetes, no granules noted.

November 19, 1913: Two cultures of each showed some contamination, bacillus.

December 9, 1913: Three cultures of each, same as on November 19.

December 15, 1913: Cultures examined, contaminating bacillus found in all.

Spirocheta suis found in 2 cultures of Hata medium, rabbit kidney, and 3 cultures of Hata medium, hog corpuscles.

Spirochetes were not numerous, but were found with little difficulty, and some were actively motile.

December 17, 1913: One of contaminated cultures of *Spirocheta suis*, examined under date of December 15, 1913, shaken in sterile water and filtered through Berkefeld. The filtrate was inoculated into plain broth, agar slant, and deep glucose agar, and after incubation under both aerobic and anaerobic conditions, no growth resulted.

December 19, 1913: Special media—(1) Hata medium with hog kidney, (2) hog serum medium, with and without hog kidney, and (3) pyrogallie serum medium (Proca, Danila, and Stroe¹) with hog kidney—were inoculated with Berkefeld filtrate of original culture, No. 653. *Spirocheta suis*.

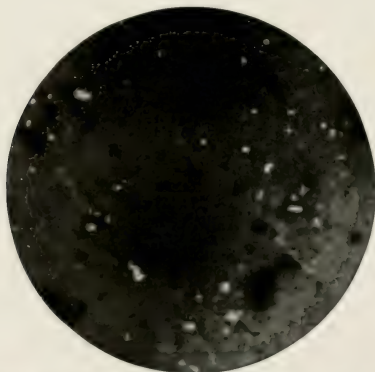


FIG. 1.—Photomicrograph of *Spirocheta suis* in culture from Berkefeld filtrate of suspension of material, ear lesion, Hog 653. $\times 1200$. (India ink preparation.)

¹Compt. rend. Soc. de biol., 1912, 72, p. 895.

DARK-FIELD EXAMINATIONS.

December 21, 1913: One culture of each medium examined. Contaminating organisms (bacilli and cocci) found. No spirochetes present.

December 24, 1913: Two cultures of each medium examined, same result as on December 21, 1913.

December 29, 1913: Three cultures of each medium examined, same result as on December 24, 1913. As all cultures in this lot appeared to be contaminated, they were discarded. From the check culture tests made, it was evident that contamination with bacilli and cocci was present in the special culture media. Repeated results on other occasions confirm the conclusion that contamination of these special media occurs as the result of adding the kidney tissue and hog corpuscles to the medium after it is prepared.

January 5, 1914: Culture 653, containing *Spirochæta suis*, first generation, described under first section of these notes, was shaken in sterile water and passed through Berkefeld filter as above. Filtrate was found to contain no organisms capable of developing on broth, agar, or deep glucose agar culture media, when incubated aerobically and anaerobically for several days.

New lot of special deep media, some containing no kidney tissue or blood clot, was inoculated with filtrate, sterile except for granules presumably related to *Spirochæta suis*.

DARK-FIELD EXAMINATIONS.

January 9, 1914: Two cultures examined, nothing detected except numerous granules in one culture.

January 12, 1914: Several cultures examined. *Spirochæta suis* found in two cultures. Not actively motile, small, consisting of about 2-3 convolutions (old cultures show spirochetes of 4-10 convolutions, average number, perhaps 6).

January 15, 1914: Third culture found to contain spirochetes, morphology similar to those noted on January 12, 1913.

From the above short series of experiments no definite conclusions should be drawn. One must recognize the fact that filtration experiments are difficult to control and many erroneous results have been recorded in the literature from time to time.

The results of the experiments outlined in this paper have been controlled by logical methods, and are suggestive of the theory that *Spirochæta suis*, at some period in its life cycle, is capable of passing through bacteria-proof filters.

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29. Biological Products—Veterinary. By Robert H. Wilson. (*American Veterinary Review*, Vol. 41, September, 1912, pp. 668-681.)

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31. Studies on Hog Cholera. By Walter E. King and Robert H. Wilson. (*Journal of Infectious Diseases*, Vol. 11, Nov., 1912, pp. 441-458.)

32. Studies on the Virus of Hog Cholera. By Walter E. King and F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 39-41.)

33. The Physiological Activity of Cannabis Sativa. By H. C. Hamilton, A. W. Lescohier and R. A. Perkins. (*Journal of the American Pharmaceutical Association*, Vol. 2, Jan., 1913, pp. 22-30.)

34. The Iodine Content of the Small, Medium and Large Thyroid Glands of Sheep, Beef and Hogs. By T. B. Aldrich. (Original Communications. Eighth International Congress of Applied Chemistry, Vol. XIX, 1912, pp. 9-14.)

35. Studies on the Virus of Hog Cholera. By Walter E. King and Robert H. Wilson. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 16, Heft 3, 1913, pp. 367-376.)

36. On the Cultivation of the *Treponema Pallidum* (*Spirochaeta Pallida*). By F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 55-67.)

37. Studies on the Gonococcus. I. By Carl C. Warden. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 93-105.)

38. Studies on the Virus of Hog Cholera. By Walter E. King, F. W. Baeslack and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 12, March, 1913, pp. 202-205.)

39. Bacillus Bronchisepticus—Its Relation to Canine Distemper. By N. S. Ferry. (*American Veterinary Review*, Vol. 43, April, 1913, pp. 16-30.)

40. Drug Influence on Extrasystoles of the Mammalian Heart. By Carey P. McCord. (*Interstate Medical Journal*, Vol. 19, Oct., 1912, pp. 870-880.)

41. The Employment of Protective Enzymes of the Blood as a Means of Extracorporeal Diagnosis. I.—Sero-Diagnosis of Pregnancy. By Carey P. McCord. (*Surgery, Gynecology and Obstetrics*, Vol. 16, April, 1913, pp. 418-421.)

42. Tribromo-tert-Butyl Alcohol, $C_4H_9OBr_3$. By T. B. Aldrich. (*Journal of the American Chemical Society*, Vol. 33, March, 1911, pp. 386-388.)

43. On Feeding Young White Rats the Posterior and the Anterior Parts of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 31, Nov., 1912, pp. 94-101.)

44. The Rationale of the Use of Adrenalin in the Treatment of Asthma. By Carey P. McCord. (*Medical Record*, Vol. 83, March 8, 1913, pp. 431-432.)

45. Standardization of Disinfectants: Some Suggested Modifications. By H. C. Hamilton and T. Ohno. (*American Journal of Public Health*, Vol. 3, June, 1913, pp. 582-588.)

46. Preventive Measures Against Equine Influenza Based on Its Bacteriology. By N. S. Ferry. (Report of the Proceedings of the United States Live Stock Association, December, 1912, p. 127.)

47. Correcting Water. By H. C. Hamilton. (*Bulletin of Pharmacy*, Vol. 27, August, 1913, pp. 330-335.)

48. Duration of Immunity Following Small-pox Vaccination. By A. W. Lescossier. (*Journal of the American Medical Association*, Vol. 66, Aug. 16, 1913, page 487-490.)

49. On Crystalline Kombe-Strophanthin. By D. H. Brauns and O. E. Closson. (*Journal of the American Pharmaceutical Association*, May, June and July, 1913, Vol. 2.)

50. A Comparative Study of Antigens for the Wassermann Reaction. By H. R. Varney and F. W. Baeslack. (*Journal of the American Medical Association*, Vol. 66, Sept. 6, 1913, pp. 754-757.)

51. The Treatment of Tetanus. By Charles T. McClintock and Willard H. Hutchings. (*Journal of Infectious Diseases*, Vol. 13, Sept., 1913, pp. 309-320.)

52. *Spirochaeta Suis*, Its Significance as a Pathogenic Organism. Studies on Hog Cholera. By Walter E. King and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 13, Nov., 1913, pp. 463-498.)

53. Time Recorder for Kymograph Tracings. By Oliver E. Closson. (*Journal of Pharmacology and Experimental Medicine*, Vol. 5, Jan., 1914, pp. 235-238.)

54. U. S. P. Menstrua. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 86, Feb., 1914, pp. 56-61.)

55. Numerical Variations of the White Blood Cells in Mice Inoculated with Transplantable Adenocarcinoma. By F. W. Baeslack. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 20, Heft 5, 1914, pp. 421-435.)

56. A Study of the Germicidal Action of the Ultraviolet Rays. By E. M. Houghton and L. Davis. (*American Journal of Public Health*, Vol. 4, March, 1914, pp. 224-240.)

57. Some Phenomena Involved in the Life History of Spirochæta Suis—Studies on Hog Cholera. By W. E. King and R. H. Drake. (*The Journal of Infectious Diseases*, Vol. 14, March, 1914, pp. 246-250.)

THE STERILIZATION OF ADRENALIN SOLUTIONS.

BY L. W. ROWE.

(From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.)

The micro-crystalline active principle of the suprarenal glands, Adrenalin, is quite stable when chemically pure. Since it is a pure principle with a definite chemical formula¹ the powder does not readily decompose when kept under ordinary conditions. However, because of its comparative insolubility in water and its great physiological activity, the preparation most suitable for therapeutic use is a 1 in 1000 solution of Adrenalin Chloride, which is an addition product formed by the action of dilute hydrochloric acid upon Adrenalin. In dilute solutions such as this, the Adrenalin Chloride is readily oxidized, if no preservative is added to the solution and it is exposed to the air. The oxidation and consequently the deterioration of the solution is recognized by the fact that the solution becomes pink, then red and finally brown in color.

Due to this tendency to deteriorate by oxidation, it is claimed that a dilute solution of Adrenalin such as a 1 in 1000 solution would not withstand sterilization by boiling since the heat would naturally tend to hasten the oxidation greatly. The solution is sterile when put on the market, but many physicians wish to make doubly sure by sterilization immediately before use.

The following experiments were carried out with the intention of determining, first, how many times a solution of Adrenalin Chloride may be sterilized in a variety of ways without deterioration, and second, the relative stability of such a solution compared with that of a solution of a synthetic product. The latter, with analogous properties and identical composition, is claimed² to be more stable than Adrenalin which is obtained from the suprarenal glands.

FIRST SERIES.

In the first experiment 12 ampoules of Adrenalin Chloride Solution 1 in 10,000 were used. The method of standardization

of the solutions which was used in this as well as in all the following experiments was the blood-pressure method,⁵ in which the solution to be tested is diluted till the Adrenalin Chloride content is 1 in 100,000. This dilution is then tested in comparison with a 1 in 100,000 solution which is made up from an accurately weighed amount of C. P. Adrenalin crystals. One ampoule in this series was used as a control and its activity found to be equal to standard. The other eleven were placed in boiling water, one ampoule being removed at the end of each 15 minutes and tested. Each was found to be of standard activity.

This experiment serves to show that the activity of Adrenalin Chloride Solution in ampoules is not impaired by sterilizing in boiling water for any period of time up to 3 hours.

SECOND SERIES.

A second lot of ampoules of Adrenalin Chloride Solution was used, one ampoule as before being reserved as a control. The others were sterilized for periods of 15 minutes each, and one was removed and tested after each sterilization.

The ampoules for further sterilization were cooled to room temperature before being sterilized again, and the process was not repeated oftener than twice a day, so that considerable time elapsed between two sterilizations.

Results were as follows:

Ampoule.	Sterilized.	Activity.
No. 1.....	Not sterilized	Standard
No. 2.....	Once (15 min.)	Standard
No. 3.....	2 times	Standard
No. 4.....	3 times	Standard
No. 5.....	4 times	Standard
No. 6.....	5 times	Standard
No. 7.....	6 times	Standard
No. 8.....	7 times	Standard
No. 9.....	8 times	90 per cent.
No. 10.....	9 times	80 per cent.
No. 11.....	10 times	80 per cent.
No. 12.....	11 times	80 per cent.

These results show that ampoules of Adrenalin Chloride Solution can be sterilized a number of times (in this series 7 times)

without any deterioration, and after that the loss of activity is gradual.

THIRD SERIES.

The next experiments give a comparison of the stability of Adrenalin Chloride Solution 1 in 1000 with that of an analogous synthetic substance of equal physiologic activity. The latter was made by treating the crystals with dilute hydrochloric acid and diluting 1 in 1000. The Adrenalin Chloride Solution used was made from C. P. Adrenalin Crystals and contained no preservative other than a slight excess of hydrochloric acid. This solution was tested and found to possess standard activity.

Three different procedures were followed in sterilizing the solutions, but the conditions were duplicated for each solution in order to obtain a direct comparison. The conditions to which the solutions were subjected will be briefly stated and the results placed in the form of a table so as to give a better opportunity for comparison.

In the first method of this series 10 c.c. of the 1 in 1000 solution to be sterilized was placed in a graduated cylinder, which was then plugged with cotton, and gradually heated in a water bath to the temperature of boiling water. This temperature was maintained for 15 minutes, and at the end of that time the solution was cooled, made up to its original volume if any loss due to evaporation had occurred, and one cubic centimeter removed for testing. This process was repeated four times.

In the second method 25 c.c. of the solution to be sterilized was placed in a tightly corked bottle, which was then partially immersed in boiling water for periods of 15 minutes each. Under these conditions there was no loss by evaporation. Each solution was submitted to four periods of sterilization and a test of its activity made after each period.

In the third method of this series 20 c.c. of the solution to be tested was placed in a small open flask and boiled over a flame for 5 minutes. After each period of boiling the loss due to evaporation, which was considerable, was made up with distilled water and the activity of the solution then determined.

The results of the above experiments were all checked and are summarized in the following table:

		Solution of Adreralin Chloride.				Solution of Synthetic Sub- stance.			
		1st ster. per cent.	2nd. per cent.	3rd. per cent.	4th. per cent.	1st ster. per cent.	2nd. per cent.	3rd. per cent.	4th. per cent.
First Method:									
Cotton plugged tube in boiling water 15 minutes at a time.	}	1. 100	100	90	80	1. 100	100	80	80
		2. 100	100	90	90				
Second Method:									
Tightly stop- pered bottle in boiling water 15 minutes at a time.)	1. 100	100	100	90	1. 100	100	70	70
		2. 100	100	90	90	2. 100	90	80	80
Third Method:									
Boiled 5 minutes over flame in open flask.)	1. 100	100	90	75	1. 100	90	80	75
		2. 100	100	100	90	2. 100	90	80	80

The results obtained show that Adrenalin Chloride Solution 1 in 1000, containing no preservative other than a slight excess of hydrochloric acid, can be sterilized at least twice by heating to the temperature of boiling water under various conditions without losing any activity. After this the loss is quite gradual, so that it probably would not be noticeable therapeutically until after the fourth or fifth sterilization. By a comparison of the results obtained with Adrenalin Chloride Solution and the solution of the synthetic substance it can be seen that the loss of activity due to sterilization occurs more quickly in the case of the latter solution, and also that the deterioration is more marked.

SUMMARY.

1. Adrenalin Chloride Solution in ampoules can be heated continuously for 3 hours to the temperature of boiling water without any loss of activity.

2. Adrenalin Chloride Solution in ampoules can be sterilized by immersion in boiling water for seven distinct periods of 15 minutes each without loss of activity.

3. Adrenalin Chloride Solution can be exposed to the air and sterilized at least twice under a variety of conditions without loss of activity.

4. Adrenalin Chloride Solution is more stable than the solu-

tion of a synthetic compound when both are subjected to the same sterilization treatment.

The results obtained disprove the statement that the stability of the synthetic exceeds that of the natural product.

REFERENCES.

¹Aldrich: *Jour. Amer. Chem. Soc.*, September, 1905.

²Stoll: *Lancet-Clinic*, June 21, 1913.

³Houghton: *Jour. Amer. Med. Assoc.*, January 18, 1902.

REPRINTS OF PUBLICATIONS FROM THE RESEARCH LABORATORY, PARKE, DAVIS & CO., DETROIT, MICH.

The present system of collecting reprints of articles published from the Research Laboratory was begun in 1912. Reprints of the following articles published subsequent to that time are available and will be sent upon request.

1. On the Administration of Diphtheria Toxin in a Collodion Sac. By E. C. L. Miller. (*Journal of Infectious Diseases*, Vol. 8, January, 1911, pp. 50-65.)

2. A Further Contribution to Our Knowledge of Insecticides—Fumigants. By Chas. T. McClintock, H. C. Hamilton and F. B. Lowe. (*Journal of the American Public Health Association*, Vol. 1, April, 1911, pp. 227-238.)

3. Duboisia Hopwoodii—A Histological Study. By Oliver A. Farwell. (Reprinted from *Merck's Report*, Vol. 20, May 1, 1911.)

4. Etiology of Canine Distemper. By Newell S. Ferry. (*Journal of Infectious Diseases*, Vol. 8, June, 1911, pp. 399-420.)

5. The Resistance of Smallpox Vaccine to the Coal-tar Disinfectants. By Chas. T. McClintock and Newell S. Ferry. (*Journal of the American Public Health Association*, Vol. 1, June, 1911, pp. 418-419.)

6. Production of Immunity with Over-Neutralized Diphtheria Toxin. By Chas. T. McClintock and Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Originale, Bd. 59, July 15, 1911, pp. 456-464.)

7. Soaps from Different Glycerides—Their Germicidal and Insecticidal Values Alone and Associated with Active Agents. By H. C. Hamilton. (*Journal of Industrial and Engineering Chemistry*, Vol. 3, August, 1911, pp. 582-584.)

8. The Sleepy Grass of New Mexico: A Histological Study. By Oliver A. Farwell. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-273.)

9. Some Observations on the Physiological Action of Sleepy Grass. By A. W. Lescohier. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-275.)

10. An Investigation of the Depressor Action of Pituitary Extracts. By Carey P. McCord. (*Archives of Internal Medicine*, Vol. 8, November, 1911, pp. 609-620.)

11. The Physiology of the Pituitary Gland and the Action of Its Extracts. By Carl J. Wiggers. (*American Journal of Medical Sciences*, Vol. 141, April, 1911, pp. 502-515.)

12. A Physiological Investigation of the Treatment of Hemoptysis. By Carl J. Wiggers. (*Archives of Internal Medicine*, Vol. 8, 1911, pp. 17-38.)

13. Notes on Catgut Sterilization: A Preliminary Report. By Willard H. Hutchings. (*Annals of Surgery*, Vol. 54, July, 1911, pp. 693-695.)

14. The Relations of Pyogenic Microorganisms to the Etiology and Treatment of Skin Diseases. By Henry Rockwell Varney. (*Ohio State Medical Journal*, December, 1911.)

15. A Micrococcus with Unusual Characteristics as a Factor in a Resistant Dermatitis Resembling Acne Vulgaris. By Henry Rockwell Varney and L. T. Clark. (*Journal of Cutaneous Diseases*, Vol. 30, February, 1912, pp. 72-78.)

16. Serum Treatment of Hemorrhage and Blood Dyscrasias. By A. W. Lescohier. (*New York Medical Journal*, Vol. 95, February 3, 1912, pp. 223-229.)

17. Further Studies on the Bacillus Bronchicanis, the Cause of Canine Distemper. By Newell S. Ferry. (*American Veterinary Review*, Vol. 41, April, 1912, pp. 77-79.)

18. The Pharmacopœial Requirements for Cannabis Sativa. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, March, 1912, pp. 200-203.)

19. The Heart Tonic Unit. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 84, March, 1912, pp. 97-103.)

20. Studies on the Etiology of Equine Influenza. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, April, 1912, pp. 185-197.)

21. A Method for the Bacteriological Standardization of Disinfectants. By Tatsuzo Ohno and H. C. Hamilton. (*American Journal of Public Health*, Vol. 2, May, 1912, pp. 331-338.)

22. Physiological Testing. By E. M. Houghton. (*American Druggist*, July and September, 1911, and January and April, 1912.)

23. Bacillus Bronchisepticus (Bronchicanis): The Cause of Distemper in Dogs and a Similar Disease in Other Animals. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, July, 1912, pp. 376-391.)

24. On Feeding Young Pups the Anterior Lobe of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 30, July, 1912, pp. 352-357.)

25. A Practical Portable Incubator. By Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Original, Bd. 65, Heft 4/5, 1912, pp. 412-413.)

26. Tobacco Extracts: Their Comparative Values as Insecticides. By W. O. Hollister. (*Journal of Economic Entomology*, Vol. 5, June, 1912, pp. 263-267.)

27. The Pharmacological Assay of Pituitary Preparations. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, October, 1912, pp. 1117-1119.)

28. Pituitary Extracts in Obstetrics and Gynecology. By A. W. Lescohier and O. E. Closson. (*Journal of the Michigan State Medical Society*, Vol. 11, October, 1912, pp. 650-657.)

29. Biological Products—Veterinary. By Robert H. Wilson. (*American Veterinary Review*, Vol. 41, September, 1912, pp. 668-681.)

30. The Isolation and Cultural Characteristics of Bacillus Acne. By Edwin M. Stanton. (*Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Original, Bd. 66, Heft 5/7, 1912, pp. 386-389.)

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32. Studies on the Virus of Hog Cholera. By Walter E. King and F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 39-41.)

33. The Physiological Activity of Cannabis Sativa. By H. C. Hamilton, A. W. Lescohier and R. A. Perkins. (*Journal of the American Pharmaceutical Association*, Vol. 2, Jan., 1913, pp. 22-30.)

34. The Iodine Content of the Small, Medium and Large Thyroid Glands of Sheep, Beef and Hogs. By T. B. Aldrich. (Original Communications, Eighth International Congress of Applied Chemistry, Vol. XIX, 1912, pp. 9-14.)

35. Studies on the Virus of Hog Cholera. By Walter E. King and Robert H. Wilson. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 16, Heft 3, 1913, pp. 367-376.)

36. On the Cultivation of the Treponema Pallidum (Spirochaeta Pallida). By F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 55-67.)

37. Studies on the Gonococcus. I. By Carl C. Warden. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 93-105.)

38. Studies on the Virus of Hog Cholera. By Walter E. King, F. W. Baeslack and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 12, March, 1913, pp. 202-205.)

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40. Drug Influence on Extrasystoles of the Mammalian Heart. By Carey P. McCord. (*Interstate Medical Journal*, Vol. 19, Oct., 1912, pp. 870-880.)

41. The Employment of Protective Enzymes of the Blood as a Means of Extracorporeal Diagnosis. I.—Sero-Diagnosis of Pregnancy. By Carey P. McCord. (*Surgery, Gynecology and Obstetrics*, Vol. 16, April, 1913, pp. 418-421.)

42. Tribromo-tert-Butyl Alcohol, $C_4H_7OBr_3$. By T. B. Aldrich. (*Journal of the American Chemical Society*, Vol. 33, March, 1911, pp. 386-388.)

43. On Feeding Young White Rats the Posterior and the Anterior Parts of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 31, Nov., 1912, pp. 94-101.)

44. The Rationale of the Use of Adrenalin in the Treatment of Asthma. By Carey P. McCord. (*Medical Record*, Vol. 83, March 8, 1913, pp. 431-432.)

45. Standardization of Disinfectants: Some Suggested Modifications. By H. C. Hamilton and T. Ohno. (*American Journal of Public Health* Vol. 3, June, 1913, pp. 582-588.)

46. Preventive Measures Against Equine Influenza Based on Its Bacteriology. By N. S. Ferry. (Report of the Proceedings of the United States Live Stock Association, December, 1912, p. 127.)

47. Correcting Water. By H. C. Hamilton. (*Bulletin of Pharmacy*, Vol. 27, August, 1913, pp. 330-335.)

48. Duration of Immunity Following Small-pox Vaccination. By A. W. Lescohier. (*Journal of the American Medical Association*, Vol. 66, Aug. 16, 1913, page 487-490.)

49. On Crystalline Kombe-Strophanthin. By D. H. Brauns and O. E. Closson. (*Journal of the American Pharmaceutical Association*, May, June and July, 1913, Vol. 2.)

50. A Comparative Study of Antigens for the Wassermann Reaction. By H. R. Varney and F. W. Baeslack. (*Journal of the American Medical Association*, Vol. 66, Sept. 6, 1913, pp. 754-757.)

51. The Treatment of Tetanus. By Charles T. McClintock and Willard H. Hutchings. (*Journal of Infectious Diseases*, Vol. 13, Sept., 1913, pp. 309-320.)

52. Spirochaeta Suis, Its Significance as a Pathogenic Organism, Studies on Hog Cholera. By Walter E. King and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 13, Nov., 1913, pp. 463-498.)

53. Time Recorder for Kymograph Tracings. By Oliver E. Closson (*Journal of Pharmacology and Experimental Medicine*, Vol. 5, Jan., 1914, pp. 235-238.)

54. U. S. P. Menstrua. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 86, Feb., 1914, pp. 56-61.)

55. Numerical Variations of the White Blood Cells in Mice Inoculated with Transplantable Adenocarcinoma. By F. W. Baeslack. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 20, Heft 5, 1914, pp. 421-435.)

56. A Study of the Germicidal Action of the Ultraviolet Rays. By E. M. Houghton and L. Davis. (*American Journal of Public Health*, Vol. 4, March, 1914, pp. 224-240.)

57. Some Phenomena Involved in the Life History of *Spirochæta Suis*—Studies on Hog Cholera. By W. E. King and R. H. Drake. (*The Journal of Infectious Diseases*. Vol. 14, March, 1914, pp. 246-250.)

58. The Sterilization of Adrenalin Solutions. By L. W. Rowe. (*American Journal of Pharmacy*, Vol. 86, April, 1914, pp. 145-149.)

INFECTION AND IMMUNITY—A REVIEW.

BY N. S. FERRY.

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1. CONDITION OF INFECTION.

Infection is the successful invasion of the body by living parasites. The broader sense of the term, however, conveys to our minds, not only the process of invasion and multiplication of the microorganisms, but also the results of their specific functions upon the tissues of the body, the pathological process. Infection, therefore, results from the entrance into the body of living, pathogenic microorganisms, and its symptoms or manifestations depend upon influences relative to invading microorganisms as well as the body which they invade. Anything modifying either the microorganisms or the host must necessarily have its effect upon the final result. There are, therefore, many conditions, some of which are recognized, that control, to a certain extent, infectious or diseased processes.

Infecting Agent.—The conditions which modify infection, as regards the infecting agent, are the *virulence*, the *number of microorganisms gaining entrance to the body* and the *mode of entrance*.

Virulence may be defined as the power of microorganisms to multiply within the body and produce disease. If the virulence is increased, if in some way the power of a certain microorganism to produce a specific disease is intensified, then the dose or the number of invading microorganisms need not necessarily be so large to produce a like infection. We have, at present, very little knowledge of Nature's way of modifying the power of microorganisms to produce disease, although in the laboratory, under experimental conditions, we may increase and decrease their virulence by several methods. The most common methods of increasing the virulence, are by passing a pure culture of a pathogenic microorganism through an animal, growing the organisms upon culture media containing unheated body fluids, such as blood, blood serum or ascitic fluid, or in a collodion sac within

the body cavity of an animal. The value of these methods depends upon the fact that the microorganisms, while developing, come in contact with some of the properties derived from the living animal, and, therefore, approach Nature as closely as possible. The virulence of the virus of rabies is increased by passing it successively through rabbits, as is also the case of many known bacteria, as the pneumococcus, streptococcus and others. The virulence of the glanders bacillus, the diphtheria bacterium and other microorganisms is strengthened by passage through guinea-pigs. On the other hand, the virulence of microorganisms may be decreased by removing them from their natural environment and regular source of food supply and growing them on artificial culture media. It is found, as a rule, that under these conditions the growth of microorganisms will gradually become more luxuriant at the expense of their virulence.

There are occasions, however, when the virulence of an organism or virus is decreased on passing through an animal. While the virus of rabies will increase on passing through rabbits, it decreases on being passed through monkeys. This is well illustrated also by the smallpox virus, which presumably decreases its virulence by passing through a heifer, and we have, as a result, a much milder infection. We cannot state this as an absolute fact as the nature of the virus of smallpox or cowpox is unknown. By making use of the cowpox virus, or the smallpox virus in its altered state, we have a means of protecting against the disease in its natural state, as the smallpox vaccine will immunize against either smallpox or cowpox.

Under ordinary conditions, given the dose large enough, and the organisms virulent enough, to produce a specific infection, the results will depend, in a large part, on the path taken by the invading microorganisms. The portals of entry, or the paths by which microorganisms gain entrance to the body, are the broken skin, the digestive tract, the respiratory tract, the genital tract and the conjunctiva. Many of the causal organisms of the acute infections are very selective in their methods, producing their specific diseases only when allowed to enter the body by certain paths. The typhoid bacillus as well as the cholera spirillum has chosen the alimentary tract as the portal of entry, and will infect the individual in no other way. The

gonococcus elects the urogenital mucous membrane, or the conjunctiva, and there is no evidence at hand to prove that specific symptoms may be produced whether through the skin, alimentary tract or respiratory tract. The tetanus bacillus produces its deadly results through the broken skin or mucous membrane, while the diphtheria bacterium prefers the mucous membrane of the pharynx, and is seldom found in any other situation.

Subject of Infection.—Pathogenic bacteria are rather underhanded in their methods of warfare, for although they are ever present, they rarely attack the body unless its natural defenses and protections are disabled or broken down; or, as we say, the resistance is lowered and the individual is susceptible. In this case, the normal equilibrium being in some way disturbed, the dormant but virulent microorganisms, taking advantage of the situation and rapidly increasing in number, produce the infection. Susceptibility or lowered resistance, or in other words, imperfect or impaired reactive powers, are, as a rule, brought about by one or more of the following conditions: fatigue, exposure to cold, heat or dampness, poor hygiene, noxious gases, drugs, trauma and operation, other diseases, improper diet, thirst and age. How these various conditions affect the normal defenses of the body we are at a loss to explain.

2. VARIETIES OF INFECTION.

The results of the growth of pathogenic bacteria within the body manifest themselves in an infinite variety of ways. After the microorganisms gain entrance to the body, they may remain at or near the point of entrance, or may find their way—by means of the blood stream, lymph channels or contiguous tissues—to some remote spot; in either situation they may multiply and produce an infection. This form of infection is termed local, and may be either acute or chronic, depending upon the length of time the morbid process has continued. Cerebrospinal meningitis, diphtheria, tetanus, the early manifestations of gonorrheal infection, as well as the ordinary pus infections, are typical local infections.

A general infection, on the other hand, is one in which the microorganisms are found disseminated throughout the entire

system. This is termed a bacteremia or a septicemia. Typhoid fever and pneumonia are typical general infections. The primary focus of the infection is to a certain extent localized (in the lungs in pneumonia and in the intestines in typhoid), but the bacteria may be isolated, in the majority of cases, from many of the internal organs as well as from the blood stream. This condition must be distinguished from that of diphtheria and tetanus (the toxemias), in which case, although the infection is localized, the toxins and not the bacteria are found diffused throughout the system, the symptoms resulting from the action of these toxins alone. It is impossible to draw a sharp line between the local and general infections, or the bacteremias and toxemias, for in many of the local infections blood cultures may show the causal organism in the circulating blood stream, and in all infections, both local and general, there is always some general intoxication produced.

A primary infection is an original or initial infection, which is often followed by a secondary. It is, as a rule, the principal infectious process invading the body at one time, although it may prove not to be the one to cause the death of the individual.

A secondary infection is a condition found when some other infectious process is implanted upon or associated with a primary infection. Secondary infections are usually caused by the pus organisms. When the susceptibility of the body, due to its lowered resistance, following the primary infection, is increased, the secondary organisms, which may have been present in the normal body in a quiescent state, begin to multiply, and cause symptoms typical to themselves.

Mixed or concurrent infections occur when two or more different bacterial species are found associated. Acute rhinitis, an ordinary "cold in the head," as it usually manifests itself, is a typical example of a mixed infection.

Terminal infections are found in the chronic organic diseases, as heart disease and Bright's disease. In such cases, one or more pathogenic microorganisms gain entrance to the body, and, encountering no resistance, due to the already decreased vitality of the body, multiply with great rapidity and produce death within a short time. The primary disease might have resulted

fatally in time, but it is the secondary infection which terminates the case. Pneumonia is one of the most frequent terminal infections. The terms "mixed" and "secondary," or "secondary" and "terminal," are often used interchangeably, for there is no hard and fast line of distinction between them, except that a terminal infection always results in death.

3. MODES OF BACTERIAL ACTION.

It is generally believed that in all infections the chief symptoms are due to the injurious nature of the products formed by the bacteria, and not to the invasion of the bacteria alone. In many cases, however, the intoxication is absolutely dependent upon the invasive power of the microorganisms, and unless this is strong enough to overcome the resistance of the body, infection will not result. On account of the poisonous nature of these products they are termed toxins. These toxins are presumably of two classes, the extracellular or exotoxins (diphtheria, tetanus) and the intracellular or endotoxins (typhoid, pneumonia and others). A few bacteria, such as the tetanus bacillus and the diphtheria bacterium, with very limited invasive powers, eliminate highly active specific toxins, which produce typical symptoms of the disease, even when separated from the bacteria. These toxins may be found in solution in the liquid culture media containing the growing microorganisms, or in the body, after being formed by the bacteria; in which case they enter the circulation and act upon parts of the body remote from the bacteria themselves. The symptoms of the diseases are due to the toxins. Unlike ordinary chemical substances, most of the true toxins act only after a definite length of time, or incubation period. Other varieties of microorganisms, with invasive powers less limited than the former, may be found growing in some localized area, but exciting actively destructive reactions in the tissues with which they come in contact. These are the staphylococci, streptococci, gonococci, meningococci, and others. Still another variety may be found either in a limited invasion, or in a general infection where the severe symptoms are also due to toxins, as in pneumonia, typhoid fever, and cholera. Lastly, we have a variety of microorganisms that depend entirely upon the general invasion before the toxin can produce

the injurious symptoms, as with the anthrax bacillus. The toxins, however, in all of the last three varieties are endotoxins. They are not found in the culture media in which the organisms grow, are isolated with difficulty from the microorganisms, and then only after their death, and do not produce symptoms of the specific disease if injected subcutaneously or otherwise, although they may produce severe symptoms and even death. The toxic power of these bacteria is intrinsic, and is dependent in great part upon their invasion.

4. NATURAL DEFENSES OF THE BODY AGAINST THE INVASION OF MICROORGANISMS.

Nature, with its unlimited resources, has endowed the human body with many wonderful and varied devices and means for defending and protecting itself against the invasion of microorganisms, and for neutralizing the deadly poisons as they are formed during the growth of these bacteria within the body.

The External Defenses.—For preventing the bacteria from entering the body we have the skin and mucous membranes. On those parts most exposed to injury, such as the soles of the feet and palms of the hands, we find the epidermis or cuticle much thicker and tougher than in other parts. The bacteria gain entrance through these parts only by means of an injury to them. For those parts of the body that are not so much exposed to external influences, and on account of their situation it is necessary that their surfaces rub together continually, nature has provided a lubricant to prevent them from erosion. The lubricants are the products of the secretions from the salivary, the gastric, the lacrimal, the mucous, and the serous glands. Aside from the protection afforded by the lubrication of the surfaces, as well as an aid in washing away the bacteria, these fluids have, to a certain extent, germicidal or at least antiseptic properties. Under normal conditions, the pathogenic microorganisms, if not killed, are checked in their growth, and often prevented from multiplying and producing disease. We are still in the dark as to the nature of these antiseptic properties. We do know, however, that although we are unable to use any of the ordinary disinfectants on these surfaces, operations upon the eye, mouth, stomach or anus are rarely followed by infection due to entrance of bacteria

at the field of operation. The respiratory tract, which is constantly invaded by foreign particles, as dust, coal and other débris carrying many hundreds of bacteria with them, is protected, not only by the mucus of the lining membrane, but also by little hair-like processes called cilia. These are continually moving in one direction, making it possible for any foreign particles, which may lodge upon the surfaces, to be gradually swept away from the lungs toward the exterior of the body.

The Internal Defenses.—A study of the internal defenses of the body brings us at once to the subject of immunity. Immunity is that condition of the body whereby it resists the development of infectious or morbid processes. The external defenses oppose the entrance and retard the invasion of microorganisms; while the internal defenses resist their development, or protect the body tissues against their poisonous products, if, by chance, the microorganisms manage to find their way past the external defenses. As we are dealing with the products of bacteria (toxins) as well as the bacteria themselves, we must distinguish between an antimicrobial and an antitoxic immunity. One is an immunity to an infection, while the other is an immunity to an intoxication. An individual may possess an immunity to a certain microorganism or to its toxin, or both, but the possession of one property does not necessarily imply the possession of the other. The inoculation of an animal with a large number of *Bacteria diphtheriæ* will not necessarily cause the appearance of unfavorable symptoms, although the injection of a very small quantity of toxin eliminated by the same strain of organisms invariably means death. On the other hand, an animal might be immunized against the endotoxin of a virulent strain of an organism such as the pneumococcus, and at the same time succumb to the injection of a small number of the live organisms.

THEORIES OF IMMUNITY.

From an early period in the history of immunity, there has been a tendency for investigators to be divided into two schools relative to the methods pursued by the body in protecting itself against the ravishes of pathogenic microorganisms; one dominated by the idea that the body fluids are chiefly concerned (the

"humoral" view), the chief supporter of which is Ehrlich; the other, championed by Metchnikoff, attributing the most important rôle to certain cells of the body (the cellular view). More recent work has shown that these views are not necessarily antagonistic, but that both seem to have their part to play in the study of the processes of immunity, and one cannot be accepted to the exclusion of the other. Both have their faults, and yet both are founded on certain demonstrable facts.

The first attempt at an explanation which gave the slightest ray of hope to the solution of the problems of acquired resistance of the body against the invasion of foreign substances was suggested by that brilliant investigator, Metchnikoff. He studied the behavior of the white blood cells, and attributed the destruction of bacteria in the body to their activities. Others had previously noted that bacteria were at times found in these cells, but Metchnikoff made an exhaustive study of the fact, and upon this founded the Theory of Phagocytosis. The cells which took up and devoured or digested the bacteria were called phagocytes, and of these he recognized two groups—the small phagocytes or white blood cells, and the large phagocytes or cells derived from the endothelial and other tissues of the body. The small phagocytes or "microphages," as Metchnikoff termed them, seem to attempt to defend the body against the acute infectious diseases, while the large phagocytes or "macrophages" pay more attention to the animal parasites, and to the microorganisms which cause chronic infections.

Ehrlich's theory, the Side Chain Theory, on the other hand, is based on the idea that the process of immunity is of a chemical nature; and that the antibody arises from the normal body cells, and has nothing to do with the phagocytes. He believes that the normal body cell consists of a central complex protoplasm, giving it definite and special properties. With the complex protoplasm are associated other combinations, similar to those in the benzol ring in chemistry, which have the function or property of combining with extracellular or outside material, and are called receptors or side chains. In performing the normal and every-day functions, as the assimilation and absorption of food after digestion, certain of these cells in the body have their part to play in the act. The assimilation is due to a chemical union between the

receptors of the cell, on the one hand, and the nutrient material on the other, brought to them by the blood and lymph from the digestive apparatus. By means of this chemical union of the receptor and nutrient material, the protoplasm of the cell is fed. As in chemistry, in order for a certain molecule to enter the ring or be assimilated by the protoplasm of the cell, it must be able to satisfy the affinity of some receptor; so in the every-day functions of the body the nutrient material must find its corresponding receptor, or side chain, before it can enter the cell. Ehrlich's theory, therefore, depends upon the supposition that toxins or poisons, like food or nutrient material, can combine with certain receptors and then enter the cell and destroy it. Ehrlich found that the property of the diphtheria toxin to combine with the antitoxin was constant, while the property of producing disease was variable, and from this was led to believe that the toxin molecule had two parts or groups—the toxic part or toxophore group, and the non-toxic part or haptophore group. He demonstrated, also, that the toxin molecule combined with certain cells, by means of this haptophore group attaching itself to the receptor of the cell. The haptophore group, therefore, is the link which attaches the toxic part of the toxin molecule to the receptor or side chain of the cell, and without it the toxin molecule would have no effect whatever upon the cell. The haptophore group, or combining group, is always constant; while the toxophore group, or disease-producing group, is variable. If the toxin, after attaching itself to the cell, damages it to any great extent, it causes the cell to die. If enough of the cells are thus damaged, the body as a whole is overcome by the poison and fatal results follow. On the other hand, if the cell is slightly affected, as is found in producing artificially active immunity, the cell in losing one receptor proceeds at once to generate and produce others. In this process of regeneration there is an overproduction of these receptors, which are cast off into the system, and find their way to the blood stream, lymph channels and possibly the tissue juices. Although these receptors are cast off from the cells, they are still able to combine with the toxin molecule. The cast-off receptors or side-chains are the antibodies or antitoxins, and by combining with toxin, either in another animal body or in the test tube, will neutralize it and produce a chem-

ically inert substance. The receptor, therefore, becomes an antitoxin as soon as it is cast off, but not until then. These receptors may remain in the individual or animal in which they are formed, and continue to protect the body. This is the active immunity we see following infectious diseases and called naturally acquired immunity, or following forced immunity, as seen in an animal immunized against the diphtheria toxin or the tetanus toxin, called the artificially acquired immunity. These receptors may be transferred to another animal body, producing a condition recognized when an individual is injected with antitoxin, either for prophylactic or curative purpose, called passive immunity. In order to satisfy other conditions of immunity, Ehrlich found it necessary to distinguish three orders of receptors. The side-chain theory, in fact, is built up on such an ingenious plan that almost any condition imaginable in immunity may be accounted for by producing other orders of receptors. In the first order we find the simple receptor, which combines with the haptophore group of the toxin molecule. In this order are placed the antitoxins. Agglutinins and precipitins are included in the receptor of the second order, and the cytolytins, including the bacteriolysins, are of the third order.

5. IMMUNITY.

We will first divide immunity into Natural, Inherited and Acquired. Natural immunity is the inherent, innate insusceptibility to disease. It is difficult, therefore, according to our present knowledge, to discriminate between natural and inherited immunity. It is a well known and recognized fact that certain species, races and individuals, under apparently the same conditions, are very resistant to some infections, and that others are extremely susceptible to the same infections. Typhoid fever, cholera, diphtheria, scarlet fever, measles, whooping-cough, mumps and other diseases occur in man only; animals are naturally immune. Some of the diseases which are found in animals alone are hog cholera (swine), contagious pleuropneumonia (cattle), equine influenza (horses and mules), and blackleg (sheep and heifers). Diseases common to both man and animals are tuberculosis, anthrax, glanders, pyemia, tetanus, plague, actinomycosis, cowpox and many others. There are, also, excep-

tions to these. The goat and dog are considered naturally immune to tuberculosis. While ordinary sheep are susceptible to anthrax, Algerian sheep are resistant. Rats are immune to anthrax, while other rodents are not. Pigeons are immune to anthrax, while most birds are susceptible. Snake venom is extremely poisonous to both man and animals, with one exception; hogs are immune. The field mouse is very susceptible to the glanders bacterium, while the white mouse is immune. Another very interesting fact which may be considered an example of natural immunity is found in the mosquito. The *Culex* does not harbor the parasite of malaria, while the *Anopheles* is its common host. The ability to transmit yellow fever is limited to one particular species of mosquito, the *Stegomyia calopus*. Individual differences in immunity, or the natural power of resistance under similar existing conditions, occur every day. In an epidemic of typhoid fever, due to a contaminated water supply, some individuals become infected while others do not; and this may occur in the same family. Again, with whooping-cough, scarlet fever, measles or any of the acute contagious diseases of childhood, one of the children in a family may become infected, while the others will remain well. The immunity is oftentimes carried through the lifetime of the individual.

Acquired Immunity.—An acquired immunity is that form of immunity gained by a susceptible individual during the life of that individual. It differs from the natural immunity, in being less certain and having a variable duration. We have two varieties of acquired immunity—naturally acquired and artificially acquired.

Naturally Acquired Immunity.—Naturally acquired immunity is established as the result of the spontaneous cure of an infectious disease. This is termed by some accidental infection.

Recovery from some of the acute infectious diseases confers a lifelong immunity to the individual, while an attack of others results in a decreased resistance. Smallpox, scarlet fever, typhoid fever, measles, whooping-cough, mumps and other infections produce an immunity which is, as a rule, lasting; although a few cases are recorded of a second and even a third attack, as the length of the immunity does not depend upon the severity of the infection. On the other hand, pneumonia and influenza not only

do not produce an immunity, but usually render the individual more liable to subsequent attacks. Some diseases, therefore, produce an increased resistance, while others an increased susceptibility.

Artificially Acquired Immunity.—Artificially acquired immunity is produced by intentional inoculation. This may be either for experimental or therapeutic purposes, but always with some definite end in view. This sort of immunity may be active or passive.

Active Immunity.—In the active form, the immunized individual gains its power of resistance by the unaided reaction of its own tissues, or in other words, it manufactures its own antibodies. In every case the immunity depends upon specific reactions on the part of the cells and tissues of the individual. The best example of this form of immunity is illustrated in the horse which has been immunized with diphtheria toxin to produce the antitoxin. The animal is injected with a dose of toxin not large enough to prove fatal, but toxic enough to produce a reaction. The horse becomes ill and recovers, the symptoms lasting but a few days. This process is repeated until the animal is able to stand an amount of toxin many hundred times the fatal dose. After the first injection something has changed in the animal, for it will not again react in the same way to the same dose. The cells or tissues of the body have acquired a new property by their own physiological activity, and this is termed active artificially acquired immunity. Such immunity is always gained at the expense, and often at the risk, of the individual acquiring it. There are many different ways of producing this form of immunity. It may be produced either by means of the bacteria themselves or their products. Experimentally we may use the live virulent, the attenuated, or the dead bacteria, but therapeutically the live virulent bacteria are not injected into the body on account of the danger attendant upon such treatment. Whenever possible, for prophylactic purposes, the attenuated organisms are preferable to the dead, because the symptoms are the result of a mild infection, calling forth more of a specific reaction on the part of the tissues and cells of the body, thereby resulting in a more lasting immunity. In preparing these attenuated organisms for the production of active immunity, the more

of their natural characteristics they are allowed to retain, with safety to the patient, the more potent will be the result.

Bacteria may be attenuated by mechanical or natural means. The mechanical methods are heat, chemicals, desiccation, and dialysis. The organisms are washed or scraped off the solid culture media, thrown down by centrifugation from the liquid culture media, or else are taken as they grow in the liquid culture media and are heated just enough to retard their growth or diminish their virulence. The tissues of the animal body in which the bacteria are growing may be allowed to undergo the same treatment. A typical example of an attenuated product of this last sort is blackleg or symptomatic anthrax vaccine. Subjecting the bacteria in a similar manner to certain chemicals or their fumes may produce the same results. We may also attenuate bacteria, while still growing in the culture media, by subjecting them to heat or chemical influence as in the production of Pasteur Anthrax Vaccine. In growing under these artificial or unnatural conditions, some sort of a change takes place in the microorganisms. They become less virulent, and when injected the resulting symptoms are atypical of the disease. The Pasteur vaccine for rabies is attenuated by desiccation. Whether this is in fact an attenuation of the organism or merely a dilution of the virus caused by the death of many of the organisms is a question. We may also attenuate or modify a virus by dialysis. In this manner the immunizing substance, whatever it may be, is allowed to remain intact at the expense of the toxin part of the virus which is apparently dialyzable. The natural method of attenuation is by passing the microorganisms through certain animals. This is rarely accomplished, but it has been done, as in the case of the passage of the rabies virus through monkeys. The gradual cessation of an epidemic after weeks or perhaps months of the most virulent types of infection is probably often the result of a natural attenuation of the causal organism.

In preparing dead bacteria for immunizing purposes, we strive to kill the microorganisms, but not destroy their products. Among these products are the endotoxins. We heat the bacteria, or cause them to come in contact with certain chemicals in the form of solutions or gases, just to the point of stopping their growth. Heat has been the generally accepted agent for killing the bac-

teria used for therapeutic purposes, although suspensions of the bacteria may be made with dilute germicides, as the coal-tar products or other antiseptics, just strong enough to kill them, but not to produce an injurious effect upon the therapeutic value of the vaccine or upon the patient receiving the treatment. Here again it must be borne in mind that the more injury to which we subject the organisms one way or another, the less will be the production of immunity. Heat being recognized as antagonistic to those functions of bacteria which have to do with the production of immunity, it behooves us to use as little heat as possible in the process of devitalizing the organisms. Suspensions of dead bacteria, especially those intended for prophylactic purposes, prepared without the aid of heat, are being recognized more and more as superior to those treated according to the original method. Therapeutically these suspensions of dead bacteria are used in dilute form, and are called bacterial vaccines or bacterins. These vaccines may be used either prophylactically to prevent the invasion of the bacteria or curatively to assist Nature in fighting an infection after the invasion of pathogenic bacteria has already been accomplished. It is claimed by some of the best authorities that the older the strain of organisms, the less will be their immunizing properties; therefore, those isolated recently from a diseased process are preferable to those grown for a considerable length of time away from their natural environments. This is known not to be true for all organisms, especially the typhoid, and it may not hold for any. That the immunizing properties or toxin-producing properties of bacteria do not go hand in hand with their disease-producing properties is an already known fact. The best toxin-producing strain of diphtheria bacilli with which we are familiar is known to be practically avirulent. If this holds true for the extracellular toxin producers, the same should pertain to the endocellular toxin producers. It is not necessary for an organism to be virulent and young in order to be a toxin producer, and, therefore, why should it have to be freshly isolated and virulent to be an immunizer?

It is also claimed that certain organisms which are allowed to remain for variable lengths of time in contact with their homologous antisera will retain their immunizing properties while losing their pathogenic properties. The process of neutralizing

the toxic properties of the organism with the antitoxin, termed sensitization, appears to attenuate the organisms without the aid of heat or chemicals.

The bacterial products, or the toxins, which are employed experimentally and practically, for the production of immunity in animals in the preparation of antisera, are the exotoxins and the endotoxins. Of the few exotoxins, only two are used to any great extent, the diphtheria and tetanus. We are unable to use these soluble toxins directly for therapeutic purposes, on account of their extremely harmful specific nature. Immunity is produced with them, as has already been explained in the case of the horse in the preparation of antitoxin serums, but this immunity is forced and at the risk of the individual immunized, so that for therapeutic purposes the treatment would be even more dangerous than the disease itself. A process somewhat analogous to sensitization of bacteria may be mentioned in this connection. It is known that active immunity may be produced by a soluble toxin such as the diphtheria toxin, so long as it has been sensitized or even over-neutralized by its own antitoxin before injection. The toxic portion of the toxin molecule is neutralized or rendered inert without apparently modifying the immunizing properties. This principle, which has lately been applied by Behring to the active prophylactic immunization of the human against diphtheria, has been used in this country several years for the production of active immunity in animals, especially the horse, and was suggested several years ago by Smith for use in the human. The idea is not original with Behring. By far the greater number of pathogenic bacteria do not produce soluble toxins in the liquid culture media in which they are grown, but do liberate poisonous products, when the bacterial cells are disintegrated. These poisonous or toxic products are termed the endotoxins, and when injected into animals, immunize them to a certain extent against the invasion of the specific microorganisms from which the endotoxins were obtained. The serum of the thus immunized animal has become antibacterial. Experiments have shown that it will kill the specific bacteria, by causing them to break up (a condition recognized as lysis) either in the animal or the test tube. It is termed a bactericidal serum with properties called bacteriolytic. Besides containing these substances

which kill bacteria, the serum has been endowed with some properties which cause the bacteria to clump together or agglutinate, called agglutinins, and others which render the bacteria more readily ingested by the phagocytes, called the opsonins, and still others which produce a precipitate of the specific or homologous bacterial proteids by means of substances called precipitins.

Active immunity is produced artificially not only with bacterial toxins, but also with animal poisons, as snake venom, spider toxin and eel serum; and plant poisons, such as those extracted from abrin, ricin, crotin and the poisonous mushrooms. From these poisons, which are soluble toxins, antitoxins may be obtained by immunizing animals with them. Of all of these animal and plant poisons, snake venom is the only one used for therapeutic purposes. By means of this, an antitoxin is produced which has marked prophylactic properties against snake bites, and is used extensively in countries where the poisonous snakes abound.

Passive Immunity.—Passive immunity is that form of immunity which depends upon defensive factors not originating in the animal protected, but artificially supplied to it. The protective material or antibody is furnished ready-made at the risk of another animal, and although the effect is secured at once, the immunity is only temporary. The best illustration is of that immunity produced by the injection of antidiphtheric serum. The diphtheria organisms are grown in bouillon to produce the diphtheria toxin. The horse is injected with this diphtheria toxin and becomes actively immunized to the toxin. The serum which contains the antibody, formed by the tissues of the horse, is injected into the patient suffering with the disease caused by the diphtheria organisms. The antibody is carried from the horse, by means of this serum, to the patient, who becomes immunized to the diphtheria toxin at once. The tissues and cells of the patient, therefore, are absolutely passive in the transaction, for the immunity is actually forced upon them. It is not the result of their physiological activity, hence the term passive immunity. The animal furnishing the defensive material must previously have been actively immunized. Passive immunity may be produced by injecting the following substances which contain defensive materials:

1. Blood serum of animals actively immunized by artificial methods.

2. Blood serum of animals actively immunized by natural methods.

Although the introduction, into susceptible animals, of blood serum of animals actively immunized by natural methods, such as those recovering from an infectious disease, raises their resistance to a certain degree, the immunity is not pronounced, nor is it lasting. The best results are obtained from those animals which are artificially immunized, especially in the production of diphtheria and tetanus antitoxin. The antitoxic serum is formed by the repeated injection of toxin during an extended period of time, while the quantity is pushed to the limit, so that the antitoxic strength or potency (antitoxin units per Cc.) of the serum is as high as possible.

Passive immunity may be of two kinds, antitoxic and antibacterial, depending upon whether the individual was immunized with antitoxic or antibacterial serum. The antibacterial serums are not as specific as the antitoxic, and therefore have not the same relative therapeutic value.

The antitoxic serums are the antidiphtheric and antitetanic. The antibacterial serums are the antigonococcic, antimeningococcic, antistreptococcic and antitubercule.

6. SPECIAL PHENOMENA OF IMMUNITY.

It has been shown that antitoxins, according to Ehrlich's scheme, are cast-off receptors of the first order, and that in order to explain other phenomena occurring in immunity, as agglutination, precipitation, cytolysis, bacteriolysis, and hemolysis, it becomes necessary to demonstrate other orders of receptors.

Agglutinins.—In 1895 Grüber and Durham discovered that when a few drops of an antibacterial serum were added to a suspension of homologous or corresponding bacteria, the microorganisms within a short time would be found in clumps. This phenomenon was termed agglutination, and was said to be due to definite bodies in the serum called agglutinins. The practical application of this reaction to the diagnosis of typhoid fever was brought out independently by Grünbaum and Widal, although

Widal was the first to publish his results. The Widal reaction, or the phenomenon of agglutination, may be produced by most of the antibacterial serums, and observed, therefore, in many of the bacterial infections, although the reaction is variable, and is more specific and definite in some than others. The cast-off receptors or agglutinins, which have to do with the formation of the clumps in the phenomenon of agglutination, have not only a haptophore affinity as in the case of receptors of the first order, but also another group, called a zymophore group. When an animal is immunized to a certain microörganism, these receptors of the second order are formed along with others, and many of them of course are liberated. These cast-off receptors or agglutinins, therefore, attach themselves to the specific bacteria by means of the haptophore affinity, and produce the agglutination by means of the zymophore group acting upon these attached microörganisms. The haptophore group selects and picks up the bacteria, while the zymophore group produces the reaction.

Precipitins.—The receptors concerned in the precipitation reaction are formed similar to those in the agglutination reaction. When the blood or serum of a human being is repeatedly injected into the peritoneal cavity of a rabbit, the serum of that rabbit acquires the property of precipitating the human serum, if mixed in the test tube. The receptors or precipitins are formed and liberated during the injections of serum into the rabbit. These receptors have a haptophore affinity which attaches itself to the serum of the human being, while the zymophore group produces the precipitation. If the serum of this rabbit is mixed with the serum of any other animal, the reaction will not take place, for this haptophore group can only attach itself to human serum, and the zymophore group can only react when the haptophore group is thus attached. The reaction is, therefore, a specific test for the presence of blood.

Cytolysins.—It has been found that when suspensions of various cells are injected into an animal, certain substances are formed in that animal and found in the serum which are injurious to cells similar to those injected. The cells injected may be bacterial cells, blood cells, epithelial cells, renal cells, hepatic cells, and others, but it must be borne in mind that the destructive

bodies are specific only for the kind of cells injected. For instance, if we inject red blood cells from a human being into a rabbit, then the serum of that rabbit will be injurious to human red blood cells but not to kidney cells or liver cells, and not red blood cells of any other species. It can be seen that in order to explain this phenomenon it becomes necessary to have a more elaborate receptor or side chain than for any of the previous combinations. At least two distinct substances are formed which react upon the cells in question, and these two substances must be able to combine with each other before the reaction can take place. These two combining substances are termed amboceptor and complement. The amboceptor, the immune body or receptor, is formed by the body cells during the process of injecting the red blood cells, bacteria or other cells, while the complement is always present, and, therefore, a normal constituent of the serum. The immune body or amboceptor, which is the cast-off receptor, must not only be able to unite with the specific cell, similar to the antitoxin of the first order, and the agglutinin of the second order, but go a step further and unite with another substance or body, the complement. It therefore must have two haptophore affinities, one for the complement and one for the specific cell. The final reaction, the destruction of the cells, is produced, however, by the complement, and although this is present in the normal serum, it cannot perform its function without the aid of the amboceptor, which is only formed by the injection of the cells.

Opsonins.—In studying the action of phagocytes on bacteria, it has been found that it is not primarily a reaction between the leucocytes and bacteria, as was thought by Metchnikoff and his school, but is a more complicated reaction. Wright and Douglas have demonstrated that certain substances in the blood serum are necessary before the reaction can take place. These substances which act upon the bacteria and prepare them for the leucocytes are called opsonins. What sort of changes the opsonins produce in the bacteria, rendering them susceptible to the action of the leucocytes, is not known.

Hektoen and Rurdiger have shown that the opsonins resemble the toxins, in that they apparently have a toxophore and haptophore.

phore group, one to unite with the bacteria and the other with the leucocytes. The relationship between the opsonins and the other immune substances, as the agglutinins and cytolytins, has practically been proven, so it appears that the opsonic theory represents a connecting link between the phagocytic theory of Metchnikoff and the side-chain theory of Ehrlich. It may possibly be demonstrated, in the future, that Ehrlich's theory is simply an aid in explaining the action of the phagocytes in infection, and that, after all, the leucocytes may have the most important part to play in the phenomena of immunity.

7. ANAPHYLAXIS AND SERUM SICKNESS.

One of the best descriptions of anaphylaxis is given by Hiss and Zinsser. "By anaphylaxis is meant the following train of phenomena: When a foreign proteid is introduced by subcutaneous, intraperitoneal, intravenous or subdural injection (or in some cases by feeding) into the animal body, after a time there will appear a specific hypersusceptibility of the animal for this proteid. After a definite interval, a second injection of the same substance, harmless in itself, will produce violent symptoms of illness and often rapid death in an animal so prepared. The phenomena are not limited to any given class of proteids, but are manifest in the case of animal, vegetable and bacterial proteids, and within certain limits are specific." A typical reaction may be seen in a guinea pig which has been injected with normal horse serum. Following an extremely small initial dose of the proteid (0.001 Cc.) the anaphylactic state usually develops after ten to fourteen days. After a large dose the time required for the development of the anaphylaxis is usually longer; it may even extend over weeks or months. Many theories have been offered for the explanation of the phenomenon of anaphylaxis, but none are at all satisfactory. Without going into the exposition of these different theories, we will take up one which seems to offer the most plausible and at the same time the simplest interpretation of all.

It has been found that the proteid molecule, on being split up by chemical methods, contains a toxic and a non-toxic substance. It has also been determined that this toxic substance is unlike the

toxin formed by the diphtheria bacillus, in that it does not produce a neutralizing body similar to antitoxin, when injected into an animal. It does seem to produce, however, a substance or body resembling the hemolysins, termed proteolysin, which is capable of breaking up any molecule of the same substance, which may be subsequently injected after a certain length of time. The hemolysins break up the red blood cells, setting free the hemoglobin, while this substance, the proteolysin, breaks up the proteid molecule, setting free the toxic portion. The toxic portion of the proteid molecule is extremely poisonous, and if enough is set free in the animal, fatal results will follow within a few minutes. By making a direct application of this theory, let us see how anaphylaxis may take place in a guinea pig. A small amount of horse serum (0.004 Cc.) is injected into a guinea pig. The proteid of this serum contains a toxic substance, which, acting upon the guinea pig, produces a body we have called proteolysin. This body does not form at once, but appears to take about ten days, and when once formed will remain in the guinea pig for the remainder of its life. Now after the required interval the guinea pig is again injected with horse serum, this time, however, with a much larger dose. The body or substance, the proteolysin, which was formed by the guinea pig following the first dose of serum, now acts upon the proteid of the second dose of serum, splitting it up at once into the toxic and non-toxic parts. On account of the large amount of serum constituting the second dose the amount of toxin set free must be relatively large, and acting upon the tissues of the guinea pig produce a train of symptoms recognized as the phenomenon of anaphylaxis. Vaughan and Wheeler of Ann Arbor were able to separate the proteid molecule into a toxic and non-toxic portion, and with the toxic portion experimentally produce symptoms similar to anaphylaxis, so it would seem that the theory so far is well founded.

The facts as regards anaphylaxis of which we are practically certain are as follows:

1. A condition of hypersusceptibility or hypersensitiveness is produced when a small quantity of proteid is injected, and a condition of immunity is produced when a sufficiently large dose is given.

2. The symptoms are more severe and specific the smaller the first or sensitizing dose, to a certain limit.

3. If a dose is given the animal some time between the first or sensitizing dose, after the first day, and the second or fatal dose, a condition of antianaphylaxis is produced which immunizes the animal to the fatal dose.

4. The condition of anaphylaxis may be transmitted from a sensitized animal to another through the serum, which is termed passive anaphylaxis.

5. The condition of anaphylaxis may be transmitted from mother to offspring.

6. Animals sensitized to one proteid are not sensitive to subsequent injections of other proteids; the reaction, therefore, being more or less specific.

7. The quantity for the second injection should be considerably larger than the first or sensitizing dose.

8. Animals recovering from the second injection are thereafter immune to the same substance.

9. After ten days following the sensitizing dose the animal is always susceptible to the second dose.

Other phenomena somewhat of this nature, which probably depend upon the principles involved in anaphylaxis, or at least in hypersensitization (allergy), are seen in the tuberculin and mallein reactions. The animal or individual is probably sensitized by the primary infection, while the injection of the mallein or tuberculin produced the typical reaction, which is slight, and which is due to the small amount of the dose. If the dose is large very severe symptoms or even death have been known to follow. Another condition or reaction, which has been compared to anaphylaxis, is that of serum-sickness. It has been known for several years that the injection of antitoxic sera in human beings is often followed by various skin eruptions, pain in the joints, swelling of the lymph glands, often albuminuria and fever. These symptoms appeared after an incubation of from two to ten days. It was also early found that these symptoms had nothing to do with the antitoxic reaction of the serum, but were dependent upon properties peculiar to the serum itself. Normal horse serum will produce the same symptoms. It seems certain that

anaphylaxis has something to do with these conditions, but we are unable to determine the exact relationship. Anaphylaxis in animals only follows the second injection, whereas serum-sickness often follows the first injection. The patient is more liable to the condition the greater the number of injections given. The size of the dose does not seem to make much difference in serum-sickness. The phenomenon of serum-sickness is seen in about 20 per cent of the cases injected with antitoxic serum. The symptoms may at times be troublesome, but fatal cases traceable to serum-sickness are unknown, or at least have not been proven. There are a few cases on record where death followed suddenly on the injection of antitoxic serum, but these are very rare, although very much magnified by opponents of serum-therapy. This condition has been termed sudden death. While it would appear, in explanation of this condition, that the fatal symptoms were traceable in some way to anaphylaxis, other suggestions have been offered which seem much more plausible, as they have been to a certain extent backed up by the post-mortem findings, such as the shock of the injection upon an already overburdened heart, or upon one suffering from the condition known as status lymphaticus.

Knowing that the larger part of the antitoxin is contained in the globulins of the serum, it has been found advantageous to use these globulins in the prophylaxis and treatment of diphtheria in place of the whole serum. As a result, the percentage of cases of serum sickness has been greatly reduced. This is explained on the assumption that as the amount of proteids injected are necessarily decreased, the chances of proteid poisoning are lessened. Although serum sickness is of less frequent occurrence than formerly, showing the relative increase in the safety of the globulin over the whole serum, yet a point has been raised as to the relative immunizing value of the globulins as compared to the whole serum, and, along the same line, the relative value of serums of high antitoxic potency as compared to those of low. It has been suggested that we do not obtain the same results, unit for unit, with serums of concentrated antitoxic content as we did when this method of specific therapeutics was first inaugurated and large quantities had to be injected due to the low antitoxic content of the serums. That is to say, some maintain

that we do not obtain relatively the same therapeutic results, from the immunity point of view, with 1 Cc. of a globulin containing 3000 units per Cc. or 3 Cc. of a serum containing 1000 units per Cc. as we did with 10 Cc. of a serum containing only 300 units per Cc. This is a point which only time and experience can answer.

The following table will compare fairly well the three conditions, according to the facts we have been able to obtain up to the present time:

<i>Anaphylaxis.</i>	<i>Serum Sickness.</i>	<i>Sudden Death.</i>
1. Condition is manifest only after second injection.	1. Condition often manifest after first injection.	1. Condition usually manifest after first injection.
2. Symptoms appear within an hour. Difficult to kill within five minutes.	2. Symptoms usually appear after an incubation of from two to ten days.	2. Symptoms appear immediately.
3. Very severe symptoms, usually resulting fatally.	3. Symptoms disappear within a few days and leave no bad results.	3. Symptoms always result fatally.
4. Fatal dose must be very large in proportion to therapeutic dose for man. 5 Cc. for a guinea-pig, corresponding to 3 pints for a man.	4. Size of dose of little importance.	4. Size of dose apparently not important.
5. Animal must be "sensitized" by a previous dose of the same proteid, with an interval of at least 10 days.	5. Previous dose apparently not necessary.	5. Previous dose apparently not necessary.
6. If a dose is given the animal some time between the first dose and the fatal or second dose, the animal will be immunized to the last dose.	6. Not known; experimental.	6. Not known.
7. The condition of anaphylaxis may be transmitted from a sensitized animal to another through the serum (Passive Anaphylaxis).	7. Not known.	7. Not known.
8. The condition of anaphylaxis may be transmitted from mother to offspring.	8. Not known; may be possible.	8. Not known.
9. Animals sensitized to one proteid are not sensitive to subsequent injections of other proteids.	9. Not known; probable.	9. Not known.
10. Animals recovering from the second injection are thereafter immune to the same substance.	10. The patient is the more liable to the condition the greater the number of injections given.	10.

8. RELATION OF BIOLOGIC PRODUCTS TO IMMUNITY.

Bacterial Vaccines (dead bacteria), active immunity.

Smallpox Vaccine (attenuated virus), active immunity.

Blackleg Vaccine (attenuated organisms), active immunity.

Anthrax Vaccine, Pasteur (attenuated organisms), active immunity.

Antitoxic Sera (blood serum), passive immunity.

Antibacterial Sera (blood serum), passive immunity.

Tuberculins (dead bacteria and bacterial products), active immunity.

Rabies Vaccine (attenuated virus), active immunity.

**REPRINTS OF PUBLICATIONS FROM THE RESEARCH
LABORATORY, PARKE, DAVIS & CO.,
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The present system of collecting reprints of articles published from the Research Laboratory was begun in 1912. Reprints of the following articles published subsequent to that time are available and will be sent upon request.

1. On the Administration of Diphtheria Toxin in a Collodion Sac. By E. C. L. Miller. (*Journal of Infectious Diseases*, Vol. 8, January, 1911, pp. 50-65.)

2. A Further Contribution to Our Knowledge of Insecticides—Fumigants. By Chas. T. McClintock, H. C. Hamilton and F. B. Lowe. (*Journal of the American Public Health Association*, Vol. 1, April, 1911, pp. 227-238.)

3. Duboisia Hopwoodii—A Histological Study. By Oliver A. Farwell. (Reprinted from *Merck's Report*, Vol. 20, May 1, 1911.)

4. Etiology of Canine Distemper. By Newell S. Ferry. (*Journal of Infectious Diseases*, Vol. 8, June, 1911, pp. 399-420.)

5. The Resistance of Smallpox Vaccine to the Coal-tar Disinfectants. By Chas. T. McClintock and Newell S. Ferry. (*Journal of the American Public Health Association*, Vol. 1, June, 1911, pp. 418-419.)

6. Production of Immunity with Over-Neutralized Diphtheria Toxin. By Chas. T. McClintock and Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Originale, Bd. 59, July 15, 1911, pp. 456-464.)

7. Soaps from Different Glycerides—Their Germicidal and Insecticidal Values Alone and Associated with Active Agents. By H. C. Hamilton. (*Journal of Industrial and Engineering Chemistry*, Vol. 3, August, 1911, pp. 582-584.)

8. The Sleepy Grass of New Mexico: A Histological Study. By Oliver A. Farwell. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-273.)

9. Some Observations on the Physiological Action of Sleepy Grass. By A. W. Leschier. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-275.)

10. An Investigation of the Depressor Action of Pituitary Extracts. By Carey P. McCord. (*Archives of Internal Medicine*, Vol. 8, November, 1911, pp. 609-620.)

11. The Physiology of the Pituitary Gland and the Action of Its Extracts. By Carl J. Wiggers. (*American Journal of Medical Sciences*, Vol. 141, April, 1911, pp. 502-515.)

12. A Physiological Investigation of the Treatment of Hemoptysis. By Carl J. Wiggers. (*Archives of Internal Medicine*, Vol. 8, 1911, pp. 17-38.)

13. Notes on Catgut Sterilization: A Preliminary Report. By Willard H. Hutchings. (*Annals of Surgery*, Vol. 54, July, 1911, pp. 693-695.)

14. The Relations of Pyogenic Microorganisms to the Etiology and Treatment of Skin Diseases. By Henry Rockwell Varney. (*Ohio State Medical Journal*, December, 1911.)

15. A Micrococcus with Unusual Characteristics as a Factor in a Resistant Dermatitis Resembling Acne Vulgaris. By Henry Rockwell Varney and L. T. Clark. (*Journal of Cutaneous Diseases*, Vol. 30, February, 1912, pp. 72-78.)

16. Serum Treatment of Hemorrhage and Blood Dyscrasias. By A. W. Lescohier. (*New York Medical Journal*, Vol. 95, February 3, 1912, pp. 223-229.)
17. Further Studies on the Bacillus Bronchicanis, the Cause of Canine Distemper. By Newell S. Ferry. (*American Veterinary Review*, Vol. 41, April, 1912, pp. 77-79.)
18. The Pharmacopœial Requirements for Cannabis Sativa. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, March, 1912, pp. 200-203.)
19. The Heart Tonic Unit. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 84, March, 1912, pp. 97-103.)
20. Studies on the Etiology of Equine Influenza. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, April, 1912, pp. 185-197.)
21. A Method for the Bacteriological Standardization of Disinfectants. By Tatsuzo Ohno and H. C. Hamilton. (*American Journal of Public Health*, Vol. 2, May, 1912, pp. 331-338.)
22. Physiological Testing. By E. M. Houghton. (*American Drug-gist*, July and September, 1911, and January and April, 1912.)
23. Bacillus Bronchisepticus (Bronchicanis): The Cause of Distemper in Dogs and a Similar Disease in Other Animals. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, July, 1912, pp. 376-391.)
24. On Feeding Young Pups the Anterior Lobe of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 30, July, 1912, pp. 352-357.)
25. A Practical Portable Incubator. By Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Original, Bd. 65, Heft 4/5, 1912, pp. 412-413.)
26. Tobacco Extracts: Their Comparative Values as Insecticides. By W. O. Hollister. (*Journal of Economic Entomology*, Vol. 5, June, 1912, pp. 263-267.)
27. The Pharmacological Assay of Pituitary Preparations. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, October, 1912, pp. 1117-1119.)
28. Pituitary Extracts in Obstetrics and Gynecology. By A. W. Lescohier and O. E. Closson. (*Journal of the Michigan State Medical Society*, Vol. 11, October, 1912, pp. 650-657.)
29. Biological Products—Veterinary. By Robert H. Wilson. (*American Veterinary Review*, Vol. 41, September, 1912, pp. 668-681.)
30. The Isolation and Cultural Characteristics of Bacillus Acne. By Edwin M. Stanton. (*Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Original, Bd. 66, Heft 5/7, 1912, pp. 386-389.)
31. Studies on Hog Cholera. By Walter E. King and Robert H. Wilson. (*Journal of Infectious Diseases*, Vol. 11, Nov., 1912, pp. 441-458.)
32. Studies on the Virus of Hog Cholera. By Walter E. King and F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 39-41.)
33. The Physiological Activity of Cannabis Sativa. By H. C. Hamilton, A. W. Lescohier and R. A. Perkins. (*Journal of the American Pharmaceutical Association*, Vol. 2, Jan., 1913, pp. 22-30.)
34. The Iodine Content of the Small, Medium and Large Thyroid Glands of Sheep, Beef and Hogs. By T. B. Aldrich. (Original Communications. Eighth International Congress of Applied Chemistry, Vol. XIX. 1912, pp. 9-14.)

35. Studies on the Virus of Hog Cholera. By Walter E. King and Robert H. Wilson. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 16, Heft 3, 1913, pp. 367-376.)

36. On the Cultivation of the Treponema Pallidum (Spirochæta Pallida). By F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 55-67.)

37. Studies on the Gonococcus, I. By Carl C. Warden. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 93-105.)

38. Studies on the Virus of Hog Cholera. By Walter E. King, F. W. Baeslack and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 12, March, 1913, pp. 202-205.)

39. Bacillus Bronchisepticus—Its Relation to Canine Distemper. By N. S. Ferry. (*American Veterinary Review*, Vol. 43, April, 1913, pp. 16-30.)

40. Drug Influence on Extrasystoles of the Mammalian Heart. By Carey P. McCord. (*Interstate Medical Journal*, Vol. 19, Oct., 1912, pp. 870-880.)

41. The Employment of Protective Enzymes of the Blood as a Means of Extracorporeal Diagnosis. I.—Sero-Diagnosis of Pregnancy. By Carey P. McCord. (*Surgery, Gynecology and Obstetrics*, Vol. 16, April, 1913, pp. 418-421.)

42. Tribromo-tert-Butyl Alcohol, $C_4H_7OBr_3$. By T. B. Aldrich. (*Journal of the American Chemical Society*, Vol. 33, March, 1911, pp. 386-388.)

43. On Feeding Young White Rats the Posterior and the Anterior Parts of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 31, Nov., 1912, pp. 94-101.)

44. The Rationale of the Use of Adrenalin in the Treatment of Asthma. By Carey P. McCord. (*Medical Record*, Vol. 83, March 8, 1913, pp. 431-432.)

45. Standardization of Disinfectants: Some Suggested Modifications. By H. C. Hamilton and T. Ohno. (*American Journal of Public Health*, Vol. 3, June, 1913, pp. 582-588.)

46. Preventive Measures Against Equine Influenza Based on Its Bacteriology. By N. S. Ferry. (Report of the Proceedings of the United States Live Stock Association, December, 1912, p. 127.)

47. Correcting Water. By H. C. Hamilton. (*Bulletin of Pharmacy*, Vol. 27, August, 1913, pp. 330-335.)

48. Duration of Immunity Following Small-pox Vaccination. By A. W. Lescohier. (*Journal of the American Medical Association*, Vol. 66, Aug. 16, 1913, page 487-490.)

49. On Crystalline Kombe-Strophanthin. By D. H. Brauns and O. E. Closson. (*Journal of the American Pharmaceutical Association*, May, June and July, 1913, Vol. 2.)

50. A Comparative Study of Antigens for the Wassermann Reaction. By H. R. Varney and F. W. Baeslack. (*Journal of the American Medical Association*, Vol. 66, Sept. 6, 1913, pp. 754-757.)

51. The Treatment of Tetanus. By Charles T. McClintock and Willard H. Hinchings. (*Journal of Infectious Diseases*, Vol. 13, Sept., 1913, pp. 309-320.)

52. Spirochæta Suis, Its Significance as a Pathogenic Organism. Studies on Hog Cholera. By Walter E. King and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 13, Nov., 1913, pp. 463-498.)

53. Time Recorder for Kymograph Tracings. By Oliver E. Closson. (*Journal of Pharmacology and Experimental Medicine*, Vol. 5, Jan., 1914, pp. 235-238.)

54. U. S. P. Menstrua. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 86, Feb., 1914, pp. 56-61.)

55. Numerical Variations of the White Blood Cells in Mice Inoculated with Transplantable Adenocarcinoma. By F. W. Baeslack. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 20, Heft 5, 1914, pp. 421-435.)

56. A Study of the Germicidal Action of the Ultraviolet Rays. By E. M. Houghton and L. Davis. (*American Journal of Public Health*, Vol. 4, March, 1914, pp. 224-240.)

57. Some Phenomena Involved in the Life History of Spirochæta Suis—Studies on Hog Cholera. By W. E. King and R. H. Drake. (*The Journal of Infectious Diseases*, Vol. 14, March, 1914, pp. 246-250.)

58. The Sterilization of Adrenalin Solutions. By L. W. Rowe. (*American Journal of Pharmacy*, Vol. 86, April, 1914, pp. 145-149.)

59. Infection and Immunity: A Review. By N. S. Ferry, Ph.B., M.D. (*Journal of the American Pharmaceutical Association*, Vol. 3, April and May, 1914.)

DISINFECTION.

What Disinfectant is the Most Generally Applicable for Clinical, Surgical and Sanitary Purposes?

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Phenol, discovered in 1834, was at first thought to be identical with wood creosote, which at that time was regarded very highly as a disinfectant. This was proved, however, to be incorrect, and while there were points of resemblance there were also several properties not common to both. Beechwood creosote is less poisonous and more germicidal than phenol proper, but it is only very slightly soluble, coagulates albumens more completely, and for various other reasons is almost discredited except for a few special purposes.

The discovery by Lister (1871) that suppuration of wounds could in almost every case be prevented by use of carbolic acid gave a great impetus to its use as a germicide and antiseptic. This work was the basis of the whole modern theory and practice relating to microorganisms, not only as they apply to Lister's special field of surgery but to that of sanitation as well.

Probably no discovery in biology has been more revolutionary and far reaching than his, since he shares with Pasteur the honor of demonstrating the connection between microorganisms and processes of infection. Lister found carbolic acid to be exceedingly efficient, and recommended it highly because of its ready adaptability to the processes of aseptic surgery. While it is fatal in 3- to 5-per-cent solution to the vegetative forms of microbial life, it is not always effective against spores in any dilution or even in pure form except at high temperatures. However, because of retaining its effectiveness in the presence of salts, acids, alkalies, and even protein, it ranks above mercuric chloride for some purposes, although under ideal conditions the latter is many times as efficient.

That portion of coal-tar oil which remains after phenol is removed was first recognized as having antiseptic value when it

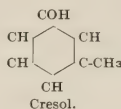
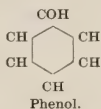
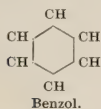
was used for pickling timber, a process patented in 1836 in England. It is still an open question what constituent of the heavy coal-tar oils is most valuable as a preservative, but its use for this purpose served to direct attention to the strongly germicidal character of certain of its constituents. A part of the oil, later known as creosote oil because of its similarity to wood creosote, was prepared as a disinfectant as early as 1874. The first preparation of importance was that known as creolin, prepared in 1887. It was analyzed, and its composition made known to the scientific world in 1889. Since that time the number of such preparations has increased enormously, and at the present time there are hundreds of the coal-tar disinfectants composed of creosote oil and soap and containing various proportions of phenols. The germicidal values of these range from that with a coefficient of 1, or about equal to pure phenol, to those of exceptional value with coefficients of 10 or more, depending on the amount and character of the phenols present.

The phenol coefficient is a term used to designate the value of a germicide. Since carbolic acid or phenol is well known as an efficient disinfectant, its value is taken as unity, and the value of any other substance similarly used can be expressed by a number called its coefficient, which indicates how many times more, or in some cases less, the disinfectant can be diluted than phenol and retain an equal germicidal value. Because of the infinite variety of combinations in coal tar, a chemical assay will not give a very accurate indication of the efficiency of an oil. Its real value can be ascertained only by actual comparison with phenol, using *B. typhosus* as the test organism, and finding the dilutions of the sample which are as efficient as certain dilutions of phenol.

These phenols are so called from their resemblance to carbolic acid or phenol proper, and are more or less closely allied to it both in composition and in properties. They differ, however, in several important characteristics. With few exceptions they are liquid and will not crystallize as carbolic acid does; they are very slightly soluble in water, and require a solvent of some character to bring them into a condition in which their activity can be measured and applied; they are much less poisonous and corrosive than phenol, and are more strongly germicidal. Chemically the phenols of ordinary coal tar differ from carbolic acid in hav-

ing one or more organic radicals attached to the benzol ring. The graphic formula for benzol is theoretically considered to be a hexagon, familiarly known as the benzol ring. Each angle of the hexagon is occupied by a CH group, and on this simple form thousands of organic compounds are built up by additions or substitution.

Phenol and cresol or cresylic acid are shown below in their generally accepted formulæ:



It is possible by synthesis to prepare derivatives still more strongly germicidal than those occurring naturally in the coal tar.

The insoluble character of the phenols and of the creosote oil containing them requires the use of an agent to be incorporated with these oils which will allow the formation of a homogeneous solution or emulsion when the product is mixed with water.

Because of the different kinds of oils used and the necessarily different treatments to prepare a substance miscible with or soluble in water, the coal-tar disinfectants differ among themselves. These differences are unimportant except from the viewpoint of efficiency, and on this basis the disinfectants may be classified into three groups:

Group 1, those with a phenol coefficient of about 2 which are soluble in soft water, to make a clear solution. Compound Solution of Cresols U. S. P. is an example of this group.

Group 2, the ordinary type of coal-tar disinfectants with coefficient of 2 to 6 which emulsify when mixed with warm, soft water.

Group 3, the high coefficient disinfectants which also emulsify when mixed with water, but which are efficient when much more highly diluted.

There are certain advantages in using these coal-tar derivatives which make them far superior to most other disinfectants. These may be summarized as follows:

Convenience.—Compared with dissolving carbolic acid crystals to make a 3- to 5-per-cent solution it is much easier to dissolve

any member of these groups. Some can be dissolved in water in any proportion; any one, if properly made, can be dissolved readily to make a stronger solution than is possible to obtain with carbolic acid crystals.

Efficiency.—The members of Group 1 will dissolve readily to make a solution of any desired strength, from that of pure carcolic acid down to one that is merely antiseptic.

The members of Group 2 are more efficient—a one- or two-per-cent solution usually far exceeding the efficiency of the strongest solution of phenol.

The members of Group 3 are still more efficient, as their phenol coefficients indicate.

Safety.—The coal-tar disinfectants are practically neither toxic nor corrosive when diluted for use on the basis of their efficiencies.

Character of Solution.—The formula for almost all the coal-tar disinfectants is such that the solution is alkaline and soapy, which aids its penetration. There is very little if any coagulation of protein to hinder penetration and lower the efficiency.

It is evident from the above comparisons that the proper use of the coal-tar disinfectants represents a distinct advance over that of carbolic acid. Even the one factor of safety alone is sufficient reason to exclude the latter from general use.

Comparing the coal-tar disinfectants with those of the metallic salts and gaseous disinfectants the most important point to be considered is:

Adaptability.—Sulphites and sulphurous acid and ferrous salts are strictly limited in their usefulness to such conditions where the abstraction of oxygen or water is detrimental or destructive to the life and development of bacteria, etc. Calcium hypochlorite and potassium permanganate are valuable where bacterial life is endangered by the action of oxygen, these substances being oxidizing agents either directly or indirectly. It is evident, therefore, that such substances are not generally applicable as disinfectants. For purifying water or sewage where oxidation of organic matter is as important as germicidal action the oxidizing agents are especially adapted.

Mercuric chloride, while being highly efficient under exceptional conditions, is so readily destroyed or so lowered in efficiency

under ordinary conditions that it is not a dependable disinfectant. It is precipitated by protein, soap and sulphur compounds; it is limited in its penetrating properties because of the coagulating action on albumen; it inhibits the growth and does not kill organisms after short exposures; it is exceedingly poisonous and irritating.

Formaldehyde is limited in its usefulness to its application as a gaseous disinfectant. In this field no preparation can replace it; but a solution of the gas in water applied as a liquid disinfectant is far inferior to phenol.

The comparisons made above indicate that even the ordinary coal-tar disinfectants when properly prepared are superior to phenol under all conditions, and surpass all other disinfectants aside from the purposes for which the latter are peculiarly adapted.

Recently coal-tar disinfectants of much higher efficiency have been prepared. The coefficients of some of these in fact are so high that the correctness of such statements is seriously questioned. The origin and chemical character of the oils from which these are prepared are more or less indefinite. Whether the character of the coal or the method of distillation by which the oils are produced is responsible for the high germicidal value is not generally known. That it is not produced in America might indicate that the character of the coal is at least partly responsible for the difference. The methods used in producing gases from coal for use in the steel and iron industries differ in England from those followed in America. It is a well-known fact that the temperature maintained and the presence or absence of air, during the destructive distillation, very markedly influence the character of the resulting products. In one case the benzols appear to oxidize to phenols, in another they appear to polymerize to naphthaline, anthracene, etc. This being true, it may be that still another series of compounds results from a slight difference in the conditions obtaining during this destructive process. The chemical composition has been claimed by Worrall (Rideal's Disinfection and Disinfectants) to be such as to place them among the organic compounds that are entirely different from the phenols of coal tar. Another writer attempts to throw

a veil of mystery over them by assigning them to the class of diphenyls.

For the purpose of this article the composition and structure of those high coefficient oils are unimportant. The sanitarian is more concerned with the efficiency of the disinfectant prepared from them. Is there real value in it in proportion to the claimed efficiency? Can confidence be placed in the statement of its value when this so far exceeds that of phenol and similar disinfectants? The question may also suggest itself, How is it possible that a product less toxic and less corrosive than carbolic acid, practically harmless when diluted as it can and should be before using, is so exceptionally active that bacteria are killed in dilutions 15 to 20 times as great as can be safely recommended for phenol?

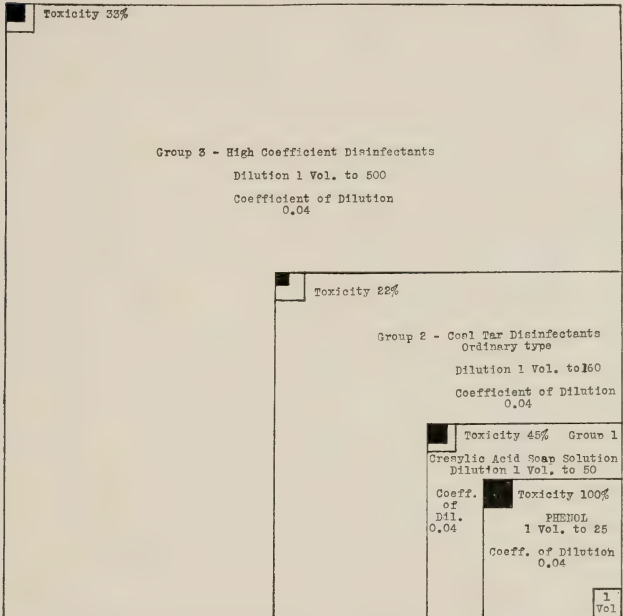
The average user of a disinfectant rarely knows from results whether it is efficient or not, because he has neither the facilities nor the skill to test it, and because incomplete disinfection or entire absence of it may not be followed by any infection directly traceable to such a failure. Even the chemist, however familiar he may be with disinfectant oils, cannot determine with certainty the efficiency of any product entirely by its chemical properties. Bacteriological rather than chemical control is, therefore, a very essential requirement for disinfectants. Hygienic measures are too vitally important to be open to suspicion regarding the substances used. The methods of applying them are so often open to question that the substances must be in the highest degree efficient.

The ideal disinfectant has been described in great detail by different authors. But the ideal disinfectant for every kind of disinfection is probably unattainable and its desirability questionable.

Formaldehyde, mercuric chloride, silver nitrate, phenol, and the coal-tar derivatives each has its sphere of usefulness, and each is more or less ideal in that sphere. Where a coal-tar derivative is applicable, however, there are certain properties possessed by this class of high coefficient disinfectants which very closely approach the ideal for general disinfection.

The average coal-tar disinfectant of Group 2 is used in a solution of one per cent, in which dilution it is about as efficient as a 3 to 5 per cent solution of phenol. One of the disinfectants of

Group 3, with a phenol coefficient of 20 when diluted to a 1-per-cent solution, is four times as efficient as a 5-per-cent solution of carbolic acid crystals. The accompanying chart is a graphic representation of the comparative efficiencies and toxicities of representatives of the three groups. The "1 Vol." square is shown in the lower right-hand corner, while the four larger



squares show the volumes to which this one volume of phenol and of each of the three groups can be diluted, all of the dilutions having the same germicidal efficiency—a phenol coefficient of 0.04.

The toxicities are shown graphically by the square in the upper left-hand corners of the four squares representing the dilutions. Phenol, being used as a standard, is entirely black, while the others are blackened in proportion to their toxicities,

expressed in per cent of that of phenol. The ratio of the black "toxicity" square is a graphic representation of the non-toxic character of these dilutions compared to that of phenol. For example, Group 3 with a toxicity of 33 per cent has a black square one-third as large as that for phenol; a comparison of the two toxicities with their corresponding dilutions shows that a solution of phenol is 60 times as toxic as a member of Group 3 diluted to an equal efficiency.

The coagulative action and the caustic and corrosive properties of the three groups can be only roughly measured; these seem, however, to be very closely associated with toxicity. So the practically harmless nature of Groups 2 and 3—particularly the latter—is evident from the chart.

Instruments are not corroded, nor is there any irritation to the healthy skin, when immersed in such a solution. The mucous membrane, however, being more sensitive will tingle and smart from application of this solution because of its alkaline and penetrating character.

Coal-tar disinfectants in general are, like phenol, unaffected by alkalis. While their values are diminished by organic matter more than is phenol, this is apparently a slower action rather than actual lowered efficiency, since there is almost no coagulation of the organic matter. In this case the fact that the disinfectant is in emulsified form explains its slow action, because necessarily the minute globules of oil composing the emulsion require a more prolonged period to penetrate moist organic matter, which tends to retard penetration.

Acids do not destroy but do materially impair the value of emulsified disinfectants in which the vehicle is soap. The same is true of hard or salty water. It is easier, however, to correct these waters or replace them with soft water with which to dilute a disinfectant than to replace soap by any other emulsifier of equal efficiency. Soap has several valuable properties in addition to that of being an efficient emulsifier of coal-tar oils. It is cleansing and tends to dissolve the natural, protective, greasy coating on the skin and other surfaces. It is not devoid of germicidal action in itself.

As a deodorant a coal-tar disinfectant has an action tending to absorb, remove, and replace bad odors. Its real value, how-

ever, is to destroy the putrefactive bacteria producing the odors—the only property which makes a deodorant of real value.

An emulsified disinfectant has been proved to exceed a soluble disinfectant even if the same constituents and the same proportion were present in each. This is shown to be due to a concentration of the minute globules of oily emulsion around the bacteria, the results being equivalent to those from a considerably stronger solution in which no minute globules of oil are present, but all is in uniform solution.

To summarize, a high coefficient disinfectant of the third group is less likely to be destroyed or inhibited in its action and is more generally applicable than the gaseous disinfectants, the oxidizing and reducing agents, or the toxic and protein-coagulating agents.

When diluted to make a solution equivalent to a 3- or 5-per-cent solution of phenol, it is

Non-toxic,

Non-corrosive to metals,

Non-coagulative to tissues,

Not caustic to the skin,

Not affected by alkalies,

Not destroyed by acids,

Not oxidized nor reduced by organic matter.

An efficient germicide,

At minimum cost per unit of efficiency.

It is not merely a substitute for carbolic acid; it is a new material possessing not only all the advantages of this valuable substance, but many others in addition. Why should the physician or the surgeon continue to use carbolic acid?

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6. Production of Immunity with Over-Neutralized Diphtheria Toxin. By Chas. T. McClintock and Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Originale, Bd. 59, July 15, 1911, pp. 456-464.)

7. Soaps from Different Glycerides—Their Germicidal and Insecticidal Values Alone and Associated with Active Agents. By H. C. Hamilton. (*Journal of Industrial and Engineering Chemistry*, Vol. 3, August, 1911, pp. 582-584.)

8. The Sleepy Grass of New Mexico: A Histological Study. By Oliver A. Farwell. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-273.)

9. Some Observations on the Physiological Action of Sleepy Grass. By A. W. Lescohier. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-275.)

10. An Investigation of the Depressor Action of Pituitary Extracts. By Carey P. McCord. (*Archives of Internal Medicine*, Vol. 8, November, 1911, pp. 609-620.)

11. The Physiology of the Pituitary Gland and the Action of Its Extracts. By Carl J. Wiggers. (*American Journal of Medical Sciences*, Vol. 141, April, 1911, pp. 502-515.)

12. A Physiological Investigation of the Treatment of Hemoptysis. By Carl J. Wiggers. (*Archives of Internal Medicine*, Vol. 8, 1911, pp. 17-38.)

13. Notes on Catgut Sterilization: A Preliminary Report. By Willard H. Hutchings. (*Annals of Surgery*, Vol. 54, July, 1911, pp. 693-695.)

14. The Relations of Pyogenic Microorganisms to the Etiology and Treatment of Skin Diseases. By Henry Rockwell Varney. (*Ohio State Medical Journal*, December, 1911.)

15. A Micrococcus with Unusual Characteristics as a Factor in a Resistant Dermatitis Resembling Acne Vulgaris. By Henry Rockwell Varney and L. T. Clark. (*Journal of Cutaneous Diseases*, Vol. 30, February, 1912, pp. 72-78.)

16. Serum Treatment of Hemorrhage and Blood Dyscrasias. By A. W. Lescohier. (*New York Medical Journal*, Vol. 95, February 3, 1912, pp. 223-229.)

17. Further Studies on the Bacillus Bronchicanis, the Cause of Canine Distemper. By Newell S. Ferry. (*American Veterinary Review*, Vol. 41, April, 1912, pp. 77-79.)

18. The Pharmacopœial Requirements for Cannabis Sativa. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, March, 1912, pp. 200-203.)

19. The Heart Tonic Unit. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 84, March, 1912, pp. 97-103.)

20. Studies on the Etiology of Equine Influenza. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, April, 1912, pp. 185-197.)

21. A Method for the Bacteriological Standardization of Disinfectants. By Tatsuzo Ohno and H. C. Hamilton. (*American Journal of Public Health*, Vol. 2, May, 1912, pp. 331-338.)

22. Physiological Testing. By E. M. Houghton. (*American Drug-gist*, July and September, 1911, and January and April, 1912.)

23. Bacillus Bronchisepticus (Bronchicanis): The Cause of Distemper in Dogs and a Similar Disease in Other Animals. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, July, 1912, pp. 376-391.)

24. On Feeding Young Pups the Anterior Lobe of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 30, July, 1912, pp. 352-357.)

25. A Practical Portable Incubator. By Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Original, Bd. 65, Heft 4/5, 1912, pp. 412-413.)

26. Tobacco Extracts: Their Comparative Values as Insecticides. By W. O. Hollister. (*Journal of Economic Entomology*, Vol. 5, June, 1912, pp. 263-267.)

27. The Pharmacological Assay of Pituitary Preparations. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, October, 1912, pp. 1117-1119.)

28. Pituitary Extracts in Obstetrics and Gynecology. By A. W. Lescohier and O. E. Closson. (*Journal of the Michigan State Medical Society*, Vol. 11, October, 1912, pp. 650-657.)

29. Biological Products—Veterinary. By Robert H. Wilson. (*American Veterinary Review*, Vol. 41, September, 1912, pp. 668-681.)

30. The Isolation and Cultural Characteristics of Bacillus Acne. By Edwin M. Stanton. (*Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Original, Bd. 66, Heft 5/7, 1912, pp. 386-389.)

31. Studies on Hog Cholera. By Walter E. King and Robert H. Wilson. (*Journal of Infectious Diseases*, Vol. 11, Nov., 1912, pp. 441-458.)

32. Studies on the Virus of Hog Cholera. By Walter E. King and F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 39-41.)

33. The Physiological Activity of Cannabis Sativa. By H. C. Hamilton, A. W. Lescohier and R. A. Perkins. (*Journal of the American Pharmaceutical Association*, Vol. 2, Jan., 1913, pp. 22-30.)

34. The Iodine Content of the Small, Medium and Large Thyroid Glands of Sheep, Beef and Hogs. By T. B. Aldrich. (Original Communications. Eighth International Congress of Applied Chemistry, Vol. XIX. 1912, pp. 9-14.)

35. Studies on the Virus of Hog Cholera. By Walter E. King and Robert H. Wilson. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 16, Heft 3, 1913, pp. 367-376.)

36. On the Cultivation of the *Treponema Pallidum* (*Spirochaeta Pallida*). By F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 55-67.)

37. Studies on the *Gonococcus*. I. By Carl C. Warden. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 93-105.)

38. Studies on the Virus of Hog Cholera. By Walter E. King, F. W. Baeslack and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 12, March, 1913, pp. 202-205.)

39. *Bacillus Bronchisepticus*—Its Relation to Canine Distemper. By N. S. Ferry. (*American Veterinary Review*, Vol. 43, April, 1913, pp. 16-30.)

40. Drug Influence on Extrasystoles of the Mammalian Heart. By Carey P. McCord. (*Interstate Medical Journal*, Vol. 19, Oct., 1912, pp. 870-880.)

41. The Employment of Protective Enzymes of the Blood as a Means of Extracorporeal Diagnosis. I.—Sero-Diagnosis of Pregnancy. By Carey P. McCord. (*Surgery, Gynecology and Obstetrics*, Vol. 16, April, 1913, pp. 418-421.)

42. Tribromo-tert-Butyl Alcohol, $C_4H_9OBr_3$. By T. B. Aldrich. (*Journal of the American Chemical Society*, Vol. 33, March, 1911, pp. 386-388.)

43. On Feeding Young White Rats the Posterior and the Anterior Parts of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 31, Nov., 1912, pp. 94-101.)

44. The Rationale of the Use of Adrenalin in the Treatment of Asthma. By Carey P. McCord. (*Medical Record*, Vol. 83, March 8, 1913, pp. 431-432.)

45. Standardization of Disinfectants: Some Suggested Modifications. By H. C. Hamilton and T. Ohno. (*American Journal of Public Health*, Vol. 3, June, 1913, pp. 582-588.)

46. Preventive Measures Against Equine Influenza Based on Its Bacteriology. By N. S. Ferry. (Report of the Proceedings of the United States Live Stock Association, December, 1912, p. 127.)

47. Correcting Water. By H. C. Hamilton. (*Bulletin of Pharmacy*, Vol. 27, August, 1913, pp. 330-335.)

48. Duration of Immunity Following Small-pox Vaccination. By A. W. Lescolier. (*Journal of the American Medical Association*, Vol. 66, Aug. 16, 1913, page 487-490.)

49. On Crystalline Kombe-Strophanthin. By D. H. Brauns and O. E. Closson. (*Journal of the American Pharmaceutical Association*, May, June and July, 1913, Vol. 2.)

50. A Comparative Study of Antigens for the Wassermann Reaction. By H. R. Varney and F. W. Baeslack. (*Journal of the American Medical Association*, Vol. 66, Sept. 6, 1913, pp. 754-757.)

51. The Treatment of Tetanus. By Charles T. McClintock and Willard H. Hutchings. (*Journal of Infectious Diseases*, Vol. 13, Sept., 1913, pp. 309-320.)

52. *Spirochaeta Suis*, Its Significance as a Pathogenic Organism, Studies on Hog Chlorea. By Walter E. King and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 13, Nov., 1913, pp. 463-498.)

53. Time Recorder for Kymograph Tracings. By Oliver E. Closson. (*Journal of Pharmacology and Experimental Medicine*, Vol. 5, Jan., 1914, pp. 235-238.)

54. U. S. P. Menstrua. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 86, Feb., 1914, pp. 56-61.)

55. Numerical Variations of the White Blood Cells in Mice Inoculated with Transplantable Adenocarcinoma. By F. W. Baeslack. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 20, Heft 5, 1914, pp. 421-435.)

56. A Study of the Germicidal Action of the Ultraviolet Rays. By E. M. Houghton and L. Davis. (*American Journal of Public Health*, Vol. 4, March, 1914, pp. 224-240.)

57. Some Phenomena Involved in the Life History of *Spirochæta Suis*—Studies on Hog Cholera. By W. E. King and R. H. Drake. (*The Journal of Infectious Diseases*, Vol. 14, March, 1914, pp. 246-250.)

58. The Sterilization of Adrenalin Solutions. By L. W. Rowe. (*American Journal of Pharmacy*, Vol. 86, April, 1914, pp. 145-149.)

59. Infection and Immunity: A Review. By N. S. Ferry, Ph.B., M.D. (*Journal of the American Pharmaceutical Association*, Vol. 3, April and May, 1914.)

60. Disinfection—What Disinfectant is the Most Generally Applicable for Clinical, Surgical and Sanitary Purposes? By H. C. Hamilton. (*Therapeutic Gazette*, Vol. 38, May, 1914, pp. 311-315.)

STUDY OF THE BACTERIOLOGY OF THE POSTERIOR NASOPHARYNX IN SCARLATINA.*

BY N. S. FERRY, M.D., DETROIT, MICH.

(From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.)

Late in 1909, at the request of Dr. E. C. Schultze, director of the Good Samaritan Dispensary of New York, the study of the bacteriology of the posterior nasopharynx in scarlatina was undertaken by the writer. He endeavored to isolate, if possible, and determine the rôle of a certain micrococcus found in this region and briefly described by Dr. Schultze in a preliminary report in the *Medical Record*, New York, Dec. 10, 1910. This organism was seen by Dr. Schultze in smears from 459 out of 555 cultures taken from the throats of patients suffering with typical symptoms of the disease.

After the writer became interested in the work, cultures were obtained, through the kindness of Dr. Schultze, from patients of the Good Samaritan Dispensary, and through the kindness of Dr. Guy L. Kiefer, of Detroit, from patients of the Herman Kiefer Hospital. On account of certain peculiarities of the organism in question, it early became evident that there were difficulties in the way of isolating and growing it in pure culture, although the organism could be seen in a large majority of cases in smears from cultures taken early in the disease. The greatest number of positive findings have been obtained by swabbing the posterior pharyngeal wall and allowing the swab to stand in a test tube of bouillon a few hours. The entire amount of bouillon is then plated in the usual manner.

The organism was not isolated in the later stages of the disease and was not found in any of the purulent discharges nor in the blood, which seems very significant, considering the fact that it appears to coincide with the contention of the majority of observers that the disease is contagious only in its early stages. All previous observations have been corroborated to the extent of finding a streptococcus predominating on the plates,

*Read before the Society of American Bacteriologists, Montreal, January 2, 1914.

while the organism next in order, with the exception perhaps of the staphylococcus, is the one fulfilling the description given by Dr. Schultze. For convenience in nomenclature this organism was called by the writer *Micrococcus* "S," and, for the present, it will continue to be designated by that term.

The *Mic.* "S" is a large coccus usually found in pairs and often in tetrads, which grows luxuriantly on all culture media after the first few generations. While the writer believes it to be a distinct species, it is distinguished at times with difficulty, morphologically, from other cocci found in the buccal cavity, both in health and disease. There is no doubt that this identical organism has been seen by other observers, yet a detailed description of an organism of a like nature has not been found in the literature. Whether the *Mic.* "S" has or has not any specific clinical or pathological significance, for the purpose of placing it on record, a detailed description of the organism will be given, followed by a brief review of the experimental work carried out with it.

Morphology.—*Mic.* "S" is a large, clearly defined, biscuit-shaped diplococcus, sometimes appearing in tetrads, measuring about the size of the meningococcus and gonococcus. It is non-spore-bearing, non-motile, non-capsulated, stains deeply with all aniline dyes and is positive to Gram. Cultural Reactions—Agar slant: Abundant, smooth, grayish-white, glistening, opaque, filiform or slightly beaded raised growth, becoming somewhat viscid within a few days. Agar deep: Abundant, filiform growth, usually in one plane, with a slightly spreading surface growth. Agar colony: Slow growing, round, smooth, convex, entire, coarsely granular colony. Bouillon: Slight growth, clear with sediment. Potato: Very slight, colorless growth. Litmus milk: No change. Koch's serum: Slight, filiform, white growth. Loeffler's serum: Abundant, filiform, smooth, glistening, pinkish-white growth. Gelatin stab: Gradual stratiform liquefaction. In about five days a cup forms at the surface and as liquefaction increases it reaches the sides of the tube and then proceeds downwards. At the end of six weeks the medium is liquefied about half-way down. Indol negative. Litmus sugars: Glucose, maltose, and saccharose gave an acid reaction; galactose, levulose and lactose, no change. Pathogenic powers: While extensive inoculation ex-

periments were carried out with this organism, nothing of any special specific significance could be gleaned from the work. Whether the organism soon loses its virulence on artificial culture media, or whether it is devoid of all pathogenic properties for the animals used, are questions which were undecided.

The following experiments were undertaken: (1) Four monkeys were inoculated subcutaneously with as many strains of *Mic. "S."* (2) Throats of four monkeys were smeared with *Mic. "S."* (3) Throats of three monkeys were swabbed with *Mic. "S."* (4) Control. Two monkeys were inoculated (Nos. 1 and 2) with streptococci taken from scarlatina throats. (5) Four monkeys were inoculated with *Mic. "S"* grown in monkey blood culture. (6) *Mic. "S"* was inoculated into mucous membrane of pharynx and also into glands of neck. These monkeys were previously given morphine subcutaneously, as a means of lowering their resistance.

In all of the experiments, with the exception of a slight rise of temperature on the fifth day, followed by a swelling of the nearest lymph glands, accompanied by a general indisposition, there were no symptoms indicative of an infection, and no signs or symptoms whatever simulating or approaching those of scarlatina as it is recognized clinically to-day. Believing, nevertheless, that this organism was found in a large enough proportion of cases to warrant further work, irrespective of the apparently negative pathogenicity, several vaccines were prepared with it which have been fairly tested out, both prophylactically and therapeutically under the supervision of Dr. Schultze in New York and Dr. Kiefer in Detroit, which work will be reported in later communications by them.

The finding of a diplococcus in such a large proportion of cases of scarlatina is suggestive of the work of Class, who, a few years ago, believed that he had discovered the cause of the disease. Class, in his original article,* described his organism as a "diplococcus, polymorphous in character, but resembling, as ordinarily seen on slides made from fresh cultures grown on any medium, a very large gonococcus." Other observers have, from time to time, noted the presence of a large diplococcus in cultures and smears from the throats of scarlatina cases, and yet no one has

**Medical Record*, 1899, Vol. LVI, p. 330.

succeeded in proving that it has any part to play in the disease, and, therefore, very little stress has been laid on the findings. The organism described by these writers is so varied in its morphology and cultural characteristics that one is inclined to one of two suppositions: either it is extremely polymorphous, as was claimed by Class, or else they were dealing with cultures containing streptococci, which organism may often be seen, in smears from the throat directly or from cultures, as large diplococci.

It is very evident, from the work of Class at any rate, that he did not always take the utmost pains to assure himself of pure cultures. He says: "I wish to state that the cultures used in these animal experiments were not isolated by means of 'Platten giessen,' but I am quite certain that they were not contaminated by other germs as repeated careful examinations showed. The reason why I did not isolate the germs by means of the process mentioned was on account of the fact that their vitality is easily destroyed by heating, the cultures obtained by isolating the germs by means of the plate method growing very poorly."

From the experience of the writer with the organism he has isolated and named *Mic. "S."* which tallies with the general descriptions given by Schultze, Class, and others, as seen in smears, it is quite essential not only to plate the cultures but to be absolutely certain that the colonies from which the cultures are taken have no small colonies of streptococci, either deep or superficial, adjacent to or near them. This can be ascertained only by examining the field with a lens. The writer employed for this work a Zeiss binocular dissecting microscope. This precaution was deemed necessary, as it was found that there appeared to be some sort of a symbiotic relationship between this organism and the streptococcus, as was evidenced by the fact that whenever this organism appeared associated with the streptococcus individual organisms were invariably seen surrounded by numerous streptococci hanging close to it.

In attempting to isolate the organism by the plate method, these streptococci are seen to have a tendency to remain in this relationship, and individual colonies of the *Mic. "S"* are seen surrounded in extremely close proximity by minute colonies of streptococci, some so small as not to be recognized by the un-

aided eye. With this experience at hand, and considering the viscid character of the growth, the writer can very readily imagine how the streptococci, unless great care was taken to avoid them, could and probably would be carried along with cultures of the micrococcus. The streptococci then would be overshadowed by the heavy growth of the organism in question, which might cause it to assume a polymorphous as well as a pathogenic character.

In pure culture, taken very carefully from single colonies some distance from colonies of streptococci, the writer has found the organism isolated by him to be extremely constant morphologically and culturally, appearing only as a large diplococcus. While Class may have had in mind this same organism, especially as seen by him in smears taken from throat cultures, the description of the strains isolated by the writer and identified by their morphology by Schultze, do not correspond with those given by Class, irrespective of the streptococcic forms mentioned by him which the writer has never seen with the *Mic. "S."* According to Class, his organism decolorized by Gram's stain, while the *Mic. "S"* retains the stain. While Class found that his organism grew sparingly on many of the ordinary culture media, necessitating the use of a special garden earth media of his own, and not at all on gelatin, the *Mic. "S"* grows fairly luxuriantly on all ordinary media, including gelatin, in which it gives a typical liquefaction. Inasmuch as it is not a certainty that Class' cultures were pure in his animal experiments, it is impossible to compare results along that line.

Among the several known organisms found in the throat, from which the *Mic. "S"* should be differentiated, the following are the most important: *Micrococcus catarrhalis*, *Micrococcus tetragenus*, *Micrococcus pharyngis siccus*, *Diplococcus intracellularis meningitidis*. Inasmuch as the *Micrococcus "S"* is Gram positive and at the same time liquefies gelatin, it will be seen that these two reactions are all that are necessary to differentiate it from any of the organisms mentioned, including the organism of Class.

The *Micrococcus catarrhalis* is Gram negative and does not liquefy gelatin, and as a rule is a much smaller organism. The *Micrococcus tetragenus* does not liquefy gelatin and is usually

found in tetrads. The *Micrococcus pharyngis siccus* is Gram negative and gives also a dry growth. The *Diplococcus intracellularis meningitidis* is both Gram negative and non-liquefying, and also gives a scanty growth on all media. The *Diplococcus scarlatinae* of Class is Gram negative, does not liquefy gelatin and grows very sparingly on all ordinary media.

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SOME EXPERIENCES WITH BACTERIAL VACCINES IN SCARLATINA.

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The bacterial vaccines employed in this work, which was carried on at the Herman Kiefer Hospital, Detroit, were as follows: A vaccine composed of streptococci isolated from the nasopharynx of individuals suffering with typical symptoms of scarlatina; a vaccine composed of an organism isolated from the same situation, called for the present *Micrococcus* "S," and described by one of us; and a vaccine composed of a mixture of the two. These vaccines which were polyvalent, being composed of several strains of each organism, contained 400 million organisms per c.c. each. Two lines of treatment were undertaken: prophylactic and curative, the nurses receiving the prophylactic treatment and the patients the curative. For curative purposes the three vaccines were used as described, while, for prophylactic purposes, the *Mic.* "S" vaccine alone was tested.

Curative tests. In order to avoid any chance of a personal factor entering into the work, the streptococcus vaccine was administered to the girls, the *Mic.* "S" vaccine to the boys, and the combined to the adults. The vaccines were given a very fair trial, extending over a period of seven months, during which time one hundred and six patients received treatment. The patients treated were proportioned as follows: Those receiving the streptococcus vaccine, twenty-nine; the *Mic.* "S" vaccine, forty-four; and the combined vaccine, thirty-three. The vaccines were given every two or three days, starting with one-half a cubic centimeter and increasing the dose as the symptoms warranted; and the treatment, as a rule, was continued until the termination of the case. No set rules for the course of treatment were adhered to; this was left entirely to the judgment of the

physician in charge. The results of the treatment will be seen in the following tabulated reports made out from the history charts:

Treated with *Mic. "S"* Vaccine.

Number of cases, 44.
 Uncomplicated, 34 (77.25 per cent.).
 Complicated, 10 (22.72 per cent.).
 Adenitis, 3.
 Adenitis and otitis media, 3.
 Purpura hemorrhagica, 1.
 Adenitis, otitis and purpura, 1.

Total.

Adenitis, 10 (22 per cent.).
 Otitis, 4 (9 per cent.).
 Purpura, 2 (4.5 per cent.).
 Deaths, 2 (4.5 per cent.).

Treated with streptococcus vaccine.

Number of cases, 29.
 Uncomplicated, 25 (86.2 per cent.).
 Complicated, 4 (13.8 per cent.).
 Otitis and adenitis, 2.
 Otitis, 2.

Total.

Adenitis, 2 (6.8 per cent.).
 Otitis, 4 (12.7 per cent.).

Treated with combined vaccine.

Number of cases, 33.
 Uncomplicated, 28 (84.8 per cent.).
 Complicated, 5 (15.15 per cent.).
 Adenitis, 1.
 Otitis, 1.
 Otitis and adenitis, 3.

Total.

Adenitis, 4 (12 per cent.).
 Otitis, 4 (12 per cent.).

Cases treated the preceding year at the hospital, without vaccines.

Number of cases, 318.
 Uncomplicated, 255 (80.19 per cent.).
 Complicated, 63 (19.81 per cent.).
 Otitis media, 21 (6.6 per cent.).
 Adenitis, 26 (8.1 per cent.).
 Nephritis, 10 (3.1 per cent.).
 Pneumonia, 3.
 Mastoid, 2.
 Arthritis, 1.
 Deaths, 14.

Comparison of the cases treated with the vaccine with cases treated according to the usual expectant methods.

Cases	Untreated, 318, per Cent.	Mic. "S" 44, per Cent.	Streptococcus 29, per Cent.	Combined 33, per Cent.
Uncomplicated	80.19	77.25	86.2	84.84
Complicated	19.81	22.72	13.8	15.15
Adenitis	8.1	22	6.8	12
Otitis media.....	6.6	9	13.7	12
Deaths	4.4	4.45	0	0

From the above résumé it would appear that the vaccines which contained the streptococci had an advantage over the ones containing the *Mic. "S"* alone, as well as over the usual expectant treatment which was previously carried on in the institution. This is what might have been expected in view of our present knowledge of the disease and experience with other diseases. Irrespective of the primary cause of the disease, it is practically conceded by all authorities that the streptococcus is a large factor in the cause of the complications, sequelæ and fatalities, and, therefore, if a vaccine has any specific action, the most favorable results would be expected with one containing this streptococcus.

Whether the *Mic. "S"* bears any relation to the disease, it would appear from the study of these few cases that, as a curative agent, a vaccine composed of this organism, alone, would not be indicated. This is also partly borne out by the fact, as pointed out by one of us in another paper, that the *Mic. "S"* is more easily isolated and is found in larger numbers in the early stages of the disease, and has not been isolated from any of the discharges.

Prophylactic tests. For prophylactic purposes the *Mic. "S"* vaccine was given in three doses of increasing strength with three to five day intervals between doses. The doses contained two hundred, four hundred, and eight hundred million bacteria respectively. Up to the time of starting this work six nurses out of about one hundred had contracted the disease. However, since the introduction of this vaccine as a prophylactic measure among all nurses who had not already had the disease, not a case has been reported. It is interesting and rather significant, also, that during a period of three months when the prophylactic treatments were discontinued, four out of about twenty nurses became infected. The apparently favorable results following the prophylactic injections may be a coincidence, but whether they are or not, the treatment is being continued and will probably be tried

in other institutions in order to test further and more conclusively the value of the vaccine.

Conclusion. It would seem from the results of the prophylactic tests with the *Mic. "S."* together with the frequency with which this organism has been found in cultures from the nasopharynx in scarlatina, that one would be warranted in giving more serious thought to it whether it has or has not up to the present given any evidence of possessing pathogenic properties of a specific nature.

**REPRINTS OF PUBLICATIONS FROM THE RESEARCH
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DETROIT, MICH.**

The present system of collecting reprints of articles published from the Research Laboratory was begun in 1912. Reprints of the following articles published subsequent to that time are available and will be sent upon request.

1. On the Administration of Diphtheria Toxin in a Collodion Sac. By E. C. L. Miller. (*Journal of Infectious Diseases*, Vol. 8, January, 1911, pp. 50-65.)

2. A Further Contribution to Our Knowledge of Insecticides—Fumigants. By Chas. T. McClintock, H. C. Hamilton and F. B. Lowe. (*Journal of the American Public Health Association*, Vol. 1, April, 1911, pp. 227-238.)

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A SERO-ENZYME TEST FOR SYPHILIS.*

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The discovery of certain substances in the gastro-intestinal canal, capable of changing the physical condition as well as chemical constitution of the food, directed attention to the processes of digestion. It soon became clear that the various ferments given off by the digestive glands were able to split the complex food molecules into less complex ones with the absorption of water. Since many of these combinations are of a colloidal nature, and, therefore, unable to pass the animal membranes, it is supposed that these substances are split up into still simpler components until their physical and chemical characteristics make them dialyzable to these membranes. Exact study of the processes of digestion as going on in the gastro-intestinal tract, brought out the fact that the highly complex molecules are broken down in the process of digestion into their component parts. Thus we find in the intestine components which result when polysaccharids, fats, albumens, and nucleoproteids are broken down with the absorption of water. This breaking down of the food is carried on until the chemical compounds have been stripped of all specific characteristics, so that a neutral grouping of the atoms results.

This process of splitting the complex chemical structures making up our food through the enzymes of the gastro-intestinal tract into their component parts can be observed *in vitro* by subjecting meat to the action of the enzymes of the stomach, pancreas and intestines. Digestion thus destroys the specific structure of the ingested food. Our food consists largely of cells, be they of animal or plant origin, which are adapted to highly specialized functions. With the function of a cell vary the proportion and chemical nature of its cell constituents. Our organism is not able to make use of such cells until they have been

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broken up into simpler compounds which, in turn, serve as starting points for various metabolic functions and for the generation of energy. The cells use these native molecules to form more complex bodies or break them down to liberate energy as it is needed.

Recent investigation concerning the constituents which make up the cell and its metabolic function demonstrates a far-reaching independence from external surroundings. The cells on the other side of the gastro-intestinal tract are, so to speak, ignorant of the nature of the food from which the native molecules were derived which enter into their building up and functional activity. Finally the cells of the liver take care of any substances which have entered the blood and are not yet suited to it. As a result of this, a nutritive substance varying within narrow limits, as to its composition, is constantly coming into contact with the cells of the body which need not be adapted to the varying conditions imposed by the varieties of food taken. The fairly constant composition of the blood furnishes them the same material from which to build the highly specialized protoplasm. The cells of the body discharge into the blood end products of their metabolism resulting from the liberation of energy and the constant building up and breaking down of the protoplasm. At first sight this would seem to change the fairly constant condition of the blood were it not for the fact that these products of normal metabolism occur in a simple form no matter by what process the reduction has taken place.

From the foregoing it is possible that under pathological conditions, certain cells will be disturbed in their metabolic activity so that substances are given off into the blood which still bear the characteristics of the cell from which they came. This may follow disturbances due to decreased cell activity or to the possible lack of ferments, or to the too rapid breaking down of the cell protoplasm. Finally, a type of cell may become so altered as to become entirely foreign to the organism and with entirely different metabolic functions.

Experiments carried out with normal sera demonstrated that they possessed no ferments capable of splitting cane sugar, peptones, or proteins. If, however, these substances were introduced parenterally it was found that they were changed. This

can be shown if the serum of an animal which has received albumen parenterally is brought into contact with the coagulated albumen in a dialyzing sac suspended in distilled water, for then the distilled water will, after a period of dialyzing, contain peptones which have been formed from the albumen. The organism is, therefore, equipped with the power of producing protective enzymes which are capable of depriving substances foreign to the blood of their specific characteristics and changing them into indifferent or native substances. The function of these enzymes in the blood is in this respect similar to the digestive ferments in the gastro-intestinal tract, in that they split the specific substances which make up our food into simple or native protein molecules capable of assimilation.

The discovery of chorionic epithelium in the blood during pregnancy by Schmorl and Veit induced Abderhalden to study the action of such blood serum on proteins. As a result of these studies (1) Abderhalden developed the method for the diagnosis of pregnancy, using placental tissue for the reaction. Freund and Abderhalden (2) extended the application of this method to the diagnosis of neoplasms. More recently this method was applied to the diagnosis of tuberculosis by J. Jessen (3), while Bundschuh and Roemer (4) as well as J. Fischer (5) made use of it in psychiatry. The scope of this preliminary communication does not permit the citing of the already extensive literature, which demonstrates the applicability of this method to many pathological conditions.

In view of these facts it seemed advisable to determine whether the serum of syphilitic patients contained such enzymes as are directed toward the *spirochæta pallida* or the cell degenerations caused by its presence in the body. If one considers the course of syphilis in its various forms, which again may differ markedly in themselves, one must realize that aside from the virulence of the infecting agent there must be present in the organism conditions which possibly are the cause of these various manifestations of the disease.

The tissue made use of in these experiments is the glistening, pearly white gummata resulting from the inoculation of rabbits with syphilitic tissue or blood of patients affected with syphilis. These resulting lesions, as has been pointed out in a previous

paper (6), are not gummata in the strict sense of the term but mucoid degeneration of the tissue. These lesions contain the spirochæta pallida in very large numbers, and practically all the original tissue has undergone degeneration. In addition, these lesions are almost free from blood and so exclude a possible factor of error in the test. By carefully trimming away any apparently sound tissue, the gummata are cut into small pieces about 4 c.mm. in size, and boiled for 10 minutes. The water is changed and the tissue boiled again for 5 minutes. This process is repeated until the water in which the tissue has been boiled no longer gives the color reaction with triketohydrindenhydrat. Then it may either be placed in a sterile bottle with the water in which it was boiled last, and to which a small amount of chloroform and toluol has been added, or the tissue may be dried *in vacuo* over sulphuric acid and ground to a fine powder, which is kept in an amber-colored glass bottle. By dipping the stopper of the bottle into toluol the ground-up tissue will be under a toluol vapor.

From analogy with other tests this reaction can also be carried out with tissue of syphilitic patients such as gummata, condylomata, and the organs of congenital syphilitic fetuses, provided these tissues can be prepared in such a way that they are free from blood.

Before undertaking the reaction, it is well to test the dialyzing thimbles. This is done with two tests. The first preliminary test serves to determine whether the dialyzing thimbles will prevent the passage of the serum albumens. Before carrying out these tests the thimbles are soaked thoroughly in running water, rinsed with distilled water. The distilled water into which the thimbles finally come is brought to a boil. This is repeated. The dialyzing thimbles are then allowed to cool in the water in which they were heated last. When sufficiently cool some chloroform and toluol is added. The thimbles are now tested with fresh unhemolyzed serum to determine whether they allow serum albumen to pass. The method of carrying out these preliminary tests is similar to the regular test, with this difference, that in this test 1 c.cm. of serum only is placed in each thimble. Those thimbles which allow the passage of protein should be discarded. It is very important that sterile, fresh unhemolyzed serum be

used, else the dialysate will give the color reaction with sound thimbles.

After the dialyzing shells have been cleaned as described above, the second preliminary test is carried out with a 0.5 per cent. aqu. sol. of Seiden peptone to determine whether the thimbles allow the passage of amino-acids during the process. Shells fulfilling these preliminary conditions may be used for the tests for quite a period, when they should be tested again for any possible flaw.

The test is carried out as follows: 8 c.cm. of sterilized filtered water are placed into the glass tubes and about 6 drops of toluol are added. Each single test requires 3 glass tubes and as many shells. The serum, which must not be more than 18 hours old, should be clear and free from any traces of hemolysis; from 0.8-1 c.cm. of serum is required. To reduce the sources of error, we again boil the tissue for 5 minutes before making the test, and test the water in which the tissue was boiled for the presence of amino-acids with triketohydrindenhydrat. The dialyzing shells are picked up with hemostats; a piece of the tissue is placed in the bottom of the first shell with clean forceps. To this is added by the use of a small, clean funnel half of the clear serum and lastly a few drops of toluol. The outside of the shell is carefully rinsed with distilled water and is then placed in the glass containing the water and toluol. The glass container is closed with a stopper to prevent evaporation. The second dialyzing shell receives the same amount of serum and toluol but no tissue, and is otherwise treated as the first; the third shell receives tissue plus an equal amount of sterile distilled water and toluol, but no serum. If more than one serum is to be tested, one control of tissue will do for all. The tubes are placed in the incubator for 12-16 hours. By carrying out this portion of the test late in the afternoon, the testing of the dialysate may be done the following morning.

After removing the dialyzing shell, 5 c.cm. of the dialysate are placed into a clean test tube and 0.2 c.cm. of 1 per cent. aqu. sol. of triketohydrindenhydrat is added, and the whole is allowed to boil actively for one minute.

The dialysate of the second tube containing serum only will frequently give a color reaction. This is due to the presence of

amino-acids in the serum. The interpretation of the test depends on the difference in the depth of color. The test is positive if the dialysate from tube one gives a darker color reaction than that of the serum control tube. The dialysate of the third tube should not give any color reaction whatever. If the color reactions of the first and second dialysate are of the same intensity or if the dialysate of the second tube gives a darker violet color than that of the first, the diagnosis is negative. The entire procedure demands careful, clean technique, which, however, is not difficult to master.

Frequently, normal serum contains dialyzable substances which will give the reaction when no tissue is added. It is evident from this that not only must such reactions be considered positive which give a dark violet color, but one must compare the reaction in the two tubes for differences in depth of color.

A positive reaction shows that the serum examined contains substances which have influenced the added protein so it has become dialyzable—that is, the serum contains proteolytic ferments. The formation of these proteolytic ferments, which are considered specific, presupposes the presence of protein not found in the serum under normal conditions. Thus a positive reaction proves: 1. The entrance into the blood of substances foreign to it. 2. The formation of a specific proteolytic ferment by the serum. The reaction disappears (1) in case of clinical cure, or when the entrance of foreign proteins into the blood stream ceases, or (2) when the organism has lost the power to produce the specific ferments in cases where the disease is still active.

The entire number of 55 sera includes 16 cases of syphilis. Eight sera came from patients in the primary stage of the disease, eighteen in the secondary stage, seven from tertiary syphilis, five from tabetics, seven from general paretics, and one from a case of congenital lues. The remaining nine sera include four from normal individuals, three from cases of chancroid, and two from scarlet fever patients.

The sera included in this communication were controlled by the clinical findings as well as by repeated Wassermann reactions. For the purpose of checking this work, samples of sera of about half the series which were studied during this investigation were

submitted to Dr. H. R. Varney, who kindly carried out the Wassermann reaction on the same. This was done before anyone was made acquainted with this test in order that independent control observation and unbiased opinions regarding the enzyme test might be recorded. I take pleasure in expressing my thanks to Dr. Varney for the assistance he has given me in connection with this work.

In addition to the three cases of chancroid, *i. e.*, 20, 25 and 46, the sera of four normal individuals and two scarlet fever patients were tested. The three cases of chancroid gave a negative sero-enzyme reaction and were also negative with the Wassermann reaction, likewise the sera of the normal individuals. The serum of scarlet fever, case 49, was positive with the ox heart antigen and negative with the foetal liver and rabbit gumma antigen, while the serum of scarlet fever case 50 gave a positive Wassermann (single +) reaction with the three above named antigens. Both sera were negative with the sero-enzyme test. Aside from these two cases, the results of the Wassermann reaction correspond with the findings of the sero-enzyme reaction in the seven remaining sera. The cerebrospinal fluid obtained from nine cases of tabes and general paresis gave a positive Wassermann reaction, while the sero-enzyme test was uniformly negative, demonstrating the absence of the enzyme in the cerebrospinal fluid. This divergence also shows that the factors entering into the Wassermann reaction are distinct from these bringing about the sero-enzyme test. The lack of these enzymes in the cerebrospinal fluid may be due to the relatively small number of white blood cells present in this fluid since the production of these enzymes may depend on these cells, for the sera from patients in this stage of the disease gave a uniformly positive sero-enzyme reaction. Case 14, presenting himself at the clinic with a recently acquired gonorrhea and giving a history of specific infection followed by prompt treatment, was free from any clinical signs of syphilis. Since both the Wassermann and sero-enzyme tests were negative, it is very probable that this patient has been cured.

The accompanying table was arranged from the histories taken at the time the patients visited the clinic.

While the Wassermann reaction at times is negative in the

WASSERMANN AND SERO-ENZYME REACTIONS

Case	Name	Sex	Age	Date in Clinic, 1913	Stage of Disease	Treatment	Wassermann R.	Sero-Enzyme Test	Amt. of Serum Used for Each Tube, c.c.
1	B. H.	♂	24	12 12	Beginning of secondary stage	None	-	+	0.5
2	M. R.	♀	19	12 12	Secondary stage	None	+	+	0.5
3	T. T.	♀	29	12 15	Primary lesion	None	-	+	0.5
4	T. W.	♀	28	12 15	Secondary stage	None	+	+	0.5
5	T. R.	♀	21	12 17	Secondary stage	0.9 gm. neosalvarsan ?	+	+	0.5
6	E. M.	♀	28	12 14	Tertiary	?	+	+	0.3
7	E. N.	♀	34	12 19	Primary lesion	None	+	+	0.5
8	W. T.	♀	39	12 19	Last secondary	0.9 gm. neosalvarsan twice	+	+	0.5
9	E. O.	♀	42	12 22	Primary lesions	None	-	+	0.5
10	F. H.	♀	32	12 23	Secondary eruption	None	+	+	0.5
11	E. O.	♀	37	12 23	Secondary eruption	Potassium iodid	+	+	0.25
12	S. H.	♀	26	12 24	Secondary eruption	?	+	+	0.5
13	J. O.	♀	24	12 26	Primary lesion	None	+	+	0.5
14	E. W.	♀	24	12 29	Secondary period (healed) ?	2 inj. intrav. of salvarsan + mercury or potassium iodid ?	-	-	0.5
15	J. H.	♀	37	12 29	Secondary lesions	?	-	+	0.5
16	E. F.	♀	24	12 30	Syphilitic anemia	2 intrav. inj. of salvarsan and potassium iodid ?	+	+	0.5
17	J. P.	♀	26	12 31 1914	Tertiary	?	+	+	0.5
18	E. W.	♀	25	1 12	Primary lesion	None ?	+	+	0.5
19	D. B.	♀	25	1 2	Secondary	?	+	+	0.5
20	St. P.	♀	32	1 5	Chancroid	+	+	0.5
21	A. S.	♀	33	1 5	Primary lesion	None ?	-	-	0.1
22	G. M.	♀	33	1 5	General paresis	?	+	+	0.5
23	M. B.	♀	34	1 5	Spec. Inf. 2 yrs. ago ; no clinical symptoms	2 intrav. inj. of neo-salv. + mercury + sod. cacodylate	+	+	0.5
24	C. A.	♀	36	1 7			+	+	0.3

25	M. S. No. 235	♂	34	1/9	Chancroid	?	-	+	0.5
26	No. 252	♂	28	1/9	Secondary eruptions	?	-	+	0.5
27	R. B.	♂	26	1/13	Late secondaries	?	-	+	0.3
28	B. C.	♂	39	1/16	Tertiary	?	-	+	0.25
29	M. S.	♂	26	1/16	Primary lesion	None	-	+	0.5
30	M. S.	♂	39	1/16	General paresis, 5 yrs.	?	-	+	0.5
31	H. D.	♂	45	1/17	Tabes	?	-	+	0.5
32	A. C.	♂	50	1/17	Tabes 6 years	?	-	+	0.5
33	K. M.	♂	54	1/17	Tabes 18 years	?	-	+	0.5
34	A. E.	♂	38	1/17	General paresis	?	-	+	0.5
35	A. C.	♂	30	1/17	General paresis	?	-	+	0.5
36	E. F.	♂	24	1/17	General paresis	?	-	+	0.5
37	M. L.	♂	24	1/17	General paresis	..	-	+	0.5
38	W. M.	♂	69	1/19	Tabes	?	-	+	0.5
39	C. M.	♂	39	1/19	Early tabes?	?	-	+	0.5
40	G. L.	♂	40	1/19	Late secondary	?	-	+	0.5
41	J. C.	♂	33	1/19	Late secondary	?	-	+	0.5
42	D. M.	♂	29	1/21	Tertiary lesion	2 inj. of neosalvarsan + mercury	-	+	0.5
43	A. K.	♂	44	1/21	Tertiary lesion	?	-	+	0.5
44	F. H.	♂	36	1/21	Early tertiary	Mercury and one inj. of neosalvarsan	-	+	0.5
45	Ed. F.	♂	25	1/23	Primary lesion	None	-	+	0.5
46	O. W.	♂	18	1/23	Chancroid	-	+	0.5
47	M. E.	♂	33	1/27	Tertiary lesion	-	+	0.5
48	A. A.	♂	26	1/27	Secondary stage	-	+	0.5
49	F. S.	♂	13	1/29	Scarlet fever	-	+	0.5
50	A. K.	♂	22	1/29	Scarlet fever	-	+	0.5
51	J. M.	♂	18	1913	Normal	-	+	0.5
52	B. B.	♂	20	12/18	Normal	-	+	0.5
53	F. B.	♂	39	12/18	Normal	-	+	0.5
54	M. S.	♂	26	1914	Normal	-	+	0.5
55	E. E.	♂	26	1/30	Congenital	-	+	0.5

* In this column ♂ denotes male and ♀ female. † Not taken. ‡ The small quantity of serum available in Case 21 may account for the result. § Spinal fluid.

primary state of syphilis, in three cases out of eight, *i.e.*, cases 3, 9, 21, in this series, the sero-enzyme reaction was positive in all. Out of the eighteen cases of secondary syphilis, four, cases 4, 12, 16, 26, gave a negative Wassermann reaction; all gave a positive sero-enzyme reaction. The specific enzyme present in the sera of syphilitics is probably directed against degenerated cell proteins rather than against the infecting agent. The sero-enzyme reaction appears to be specific and demonstrable at an earlier period than the complement fixation test since the production of specific enzymes can be stimulated by minute quantities of protein foreign to the blood, and because these proteins are constantly finding their way into the circulation in the course of this disease.

The Wassermann reactions have been carried out with three antigens simultaneously and in most instances they have been repeated for the purpose of double checking the results.

I take pleasure in expressing my thanks to Dr. Hitchcock, for supplying me with cerebrospinal fluid from tabetics and paretics, Dr. Wm. E. Keane, Dr. F. L. Clark, and Dr. G. L. Kiefer for giving me access to their clinics.

**REPRINTS OF PUBLICATIONS FROM THE RESEARCH
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The present system of collecting reprints of articles published from the Research Laboratory was begun in 1912. Reprints of the following articles published subsequent to that time are available and will be sent upon request.

1. On the Administration of Diphtheria Toxin in a Collodion Sac. By E. C. L. Miller. (*Journal of Infectious Diseases*, Vol. 8, January, 1911, pp. 50-65.)
2. A Further Contribution to Our Knowledge of Insecticides—Fumigants. By Chas. T. McClintock, H. C. Hamilton and F. B. Lowe. (*Journal of the American Public Health Association*, Vol. 1, April, 1911, pp. 227-238.)
3. Duboisia Hopwoodii—A Histological Study. By Oliver A. Farwell. (Reprinted from *Merck's Report*, Vol. 20, May 1, 1911.)
4. Etiology of Canine Distemper. By Newell S. Ferry. (*Journal of Infectious Diseases*, Vol. 8, June, 1911, pp. 399-420.)
5. The Resistance of Smallpox Vaccine to the Coal-tar Disinfectants. By Chas. T. McClintock and Newell S. Ferry. (*Journal of the American Public Health Association*, Vol. 1, June, 1911, pp. 418-419.)
6. Production of Immunity with Over-Neutralized Diphtheria Toxin. By Chas. T. McClintock and Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Originale, Bd. 59, July 15, 1911, pp. 456-464.)
7. Soaps from Different Glycerides—Their Germicidal and Insecticidal Values Alone and Associated with Active Agents. By H. C. Hamilton. (*Journal of Industrial and Engineering Chemistry*, Vol. 3, August, 1911, pp. 582-584.)
8. The Sleepy Grass of New Mexico: A Histological Study. By Oliver A. Farwell. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-273.)
9. Some Observations on the Physiological Action of Sleepy Grass. By A. W. Leschier. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-275.)
10. An Investigation of the Depressor Action of Pituitary Extracts. By Carey P. McCord. (*Archives of Internal Medicine*, Vol. 8, November, 1911, pp. 609-620.)
11. The Physiology of the Pituitary Gland and the Action of Its Extracts. By Carl J. Wiggers. (*American Journal of Medical Sciences*, Vol. 141, April, 1911, pp. 502-515.)
12. A Physiological Investigation of the Treatment of Hemoptysis. By Carl J. Wiggers. (*Archives of Internal Medicine*, Vol. 8, 1911, pp. 17-38.)
13. Notes on Catgut Sterilization: A Preliminary Report. By Wilard H. Hutchings. (*Annals of Surgery*, Vol. 54, July, 1911, pp. 693-695.)
14. The Relations of Pyogenic Microorganisms to the Etiology and Treatment of Skin Diseases. By Henry Rockwell Varney. (*Ohio State Medical Journal*, December, 1911.)
15. A Micrococcus with Unusual Characteristics as a Factor in a Resistant Dermatitis Resembling Acne Vulgaris. By Henry Rockwell Varney and L. T. Clark. (*Journal of Cutaneous Diseases*, Vol. 30, February, 1912, pp. 72-78.)

16. Serum Treatment of Hemorrhage and Blood Dyscrasias. By A. W. Lescohier. (*New York Medical Journal*, Vol. 95, February 3, 1912, pp. 223-229.)

17. Further Studies on the Bacillus Bronchicanis, the Cause of Canine Distemper. By Newell S. Ferry. (*American Veterinary Review*, Vol. 41, April, 1912, pp. 77-79.)

18. The Pharmacopœial Requirements for Cannabis Sativa. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, March, 1912, pp. 200-203.)

19. The Heart Tonic Unit. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 84, March, 1912, pp. 97-103.)

20. Studies on the Etiology of Equine Influenza. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, April, 1912, pp. 185-197.)

21. A Method for the Bacteriological Standardization of Disinfectants. By Tatsuzo Ohno and H. C. Hamilton. (*American Journal of Public Health*, Vol. 2, May, 1912, pp. 331-338.)

22. Physiological Testing. By E. M. Houghton. (*American Druggist*, July and September, 1911, and January and April, 1912.)

23. Bacillus Bronchisepticus (Bronchicanis): The Cause of Distemper in Dogs and a Similar Disease in Other Animals. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, July, 1912, pp. 376-391.)

24. On Feeding Young Pups the Anterior Lobe of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 30, July, 1912, pp. 352-357.)

25. A Practical Portable Incubator. By Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Original, Bd. 65, Heft 4/5, 1912, pp. 412-413.)

26. Tobacco Extracts: Their Comparative Values as Insecticides. By W. O. Hollister. (*Journal of Economic Entomology*, Vol. 5, June, 1912, pp. 263-267.)

27. The Pharmacological Assay of Pituitary Preparations. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, October, 1912, pp. 1117-1119.)

28. Pituitary Extracts in Obstetrics and Gynecology. By A. W. Lescohier and O. E. Closson. (*Journal of the Michigan State Medical Society*, Vol. 11, October, 1912, pp. 650-657.)

29. Biological Products—Veterinary. By Robert H. Wilson. (*American Veterinary Review*, Vol. 41, September, 1912, pp. 668-681.)

30. The Isolation and Cultural Characteristics of Bacillus Acne. By Edwin M. Stanton. (*Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Original, Bd. 66, Heft 5/7, 1912, pp. 386-389.)

31. Studies on Hog Cholera. By Walter E. King and Robert H. Wilson. (*Journal of Infectious Diseases*, Vol. 11, Nov., 1912, pp. 441-458.)

32. Studies on the Virus of Hog Cholera. By Walter E. King and F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 39-41.)

33. The Physiological Activity of Cannabis Sativa. By H. C. Hamilton, A. W. Lescohier and R. A. Perkins. (*Journal of the American Pharmaceutical Association*, Vol. 2, Jan., 1913, pp. 22-30.)

34. The Iodine Content of the Small, Medium and Large Thyroid Glands of Sheep, Beef and Hogs. By T. B. Aldrich. (Original Communications. Eighth International Congress of Applied Chemistry, Vol. XIX, 1912, pp. 9-14.)

35. Studies on the Virus of Hog Cholera. By Walter E. King and Robert H. Wilson. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 16, Heft 3, 1913, pp. 367-376.)

36. On the Cultivation of the Treponema Pallidum (Spirochæta Pallida). By F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 55-67.)

37. Studies on the Gonococcus. I. By Carl C. Warden. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 93-105.)

38. Studies on the Virus of Hog Cholera. By Walter E. King, F. W. Baeslack and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 12, March, 1913, pp. 202-205.)

39. Bacillus Bronchisepticus—Its Relation to Canine Distemper. By N. S. Ferry. (*American Veterinary Review*, Vol. 43, April, 1913, pp. 16-30.)

40. Drug Influence on Extrasystoles of the Mammalian Heart. By Carey P. McCord. (*Interstate Medical Journal*, Vol. 19, Oct., 1912, pp. 870-880.)

41. The Employment of Protective Enzymes of the Blood as a Means of Extracorporeal Diagnosis. I.—Sero-Diagnosis of Pregnancy. By Carey P. McCord. (*Surgery, Gynecology and Obstetrics*, Vol. 16, April, 1913, pp. 418-421.)

42. Tribromo-tert-Butyl Alcohol, $C_4H_7OBr_3$. By T. B. Aldrich. (*Journal of the American Chemical Society*, Vol. 33, March, 1911, pp. 386-388.)

43. On Feeding Young White Rats the Posterior and the Anterior Parts of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 31, Nov., 1912, pp. 94-101.)

44. The Rationale of the Use of Adrenalin in the Treatment of Asthma. By Carey P. McCord. (*Medical Record*, Vol. 83, March 8, 1913, pp. 431-432.)

45. Standardization of Disinfectants: Some Suggested Modifications. By H. C. Hamilton and T. Ohno. (*American Journal of Public Health*, Vol. 3, June, 1913, pp. 582-588.)

46. Preventive Measures Against Equine Influenza Based on Its Bacteriology. By N. S. Ferry. (Report of the Proceedings of the United States Live Stock Association, December, 1912, p. 127.)

47. Correcting Water. By H. C. Hamilton. (*Bulletin of Pharmacy*, Vol. 27, August, 1913, pp. 330-335.)

48. Duration of Immunity Following Small-pox Vaccination. By A. W. Lescohier. (*Journal of the American Medical Association*, Vol. 66, Aug. 16, 1913, page 487-490.)

49. On Crystalline Kombe-Strophanthin. By D. H. Brauns and O. E. Closson. (*Journal of the American Pharmaceutical Association*, May, June and July, 1913, Vol. 2.)

50. A Comparative Study of Antigens for the Wassermann Reaction. By H. R. Varney and F. W. Baeslack. (*Journal of the American Medical Association*, Vol. 66, Sept. 6, 1913, pp. 754-757.)

51. The Treatment of Tetanus. By Charles T. McClintock and Willard H. Hutchings. (*Journal of Infectious Diseases*, Vol. 13, Sept., 1913, pp. 309-320.)

52. Spirochæta Suis, Its Significance as a Pathogenic Organism, Studies on Hog Chlorea. By Walter E. King and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 13, Nov., 1913, pp. 463-498.)

53. Time Recorder for Kymograph Tracings. By Oliver E. Closson. (*Journal of Pharmacology and Experimental Medicine*, Vol. 5, Jan., 1914, pp. 235-238.)

54. U. S. P. Menstrua. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 86, Feb., 1914, pp. 56-61.)

55. Numerical Variations of the White Blood Cells in Mice Inoculated with Transplantable Adenocarcinoma. By F. W. Baeslack. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 20, Heft 5, 1914, pp. 421-435.)

56. A Study of the Germicidal Action of the Ultraviolet Rays. By E. M. Houghton and L. Davis. (*American Journal of Public Health*, Vol. 4, March, 1914, pp. 224-240.)

57. Some Phenomena Involved in the Life History of Spirochæta Suis—Studies on Hog Cholera. By W. E. King and R. H. Drake. (*The Journal of Infectious Diseases*, Vol. 14, March, 1914, pp. 246-250.)

58. The Sterilization of Adrenalin Solutions. By L. W. Rowe. (*American Journal of Pharmacy*, Vol. 86, April, 1914, pp. 145-149.)

59. Infection and Immunity: A Review. By N. S. Ferry, Ph.B., M.D. (*Journal of the American Pharmaceutical Association*, Vol. 3, April and May, 1914.)

60. Disinfection—What Disinfectant is the Most Generally Applicable for Clinical, Surgical and Sanitary Purposes? By H. C. Hamilton. (*Therapeutic Gazette*, Vol. 38, May, 1914, pp. 311-315.)

61. Study of the Bacteriology of the Posterior Nasopharynx in Scarlatina. By N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, pp. 934-935.)

62. Some Experiences with Bacterial Vaccines in Scarlatina. By Guy I. Kiefer, M.D., D.P.H., and N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, p. 936.)

63. A Sero-enzyme Test for Syphilis. By F. W. Baeslack, M.A., M.D. (*The Urologic and Cutaneous Review*, Vol. 18, May, 1914, pp. 234-238.)

BACTERIOLOGY AND CONTROL OF ACUTE INFECTIONS IN LABORATORY ANIMALS.¹

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In July, 1910, the writer published an article (1910¹) entitled "A Preliminary Report of the Bacterial Findings in Canine Distemper," in which was described the organism responsible for the disease. Within a few months a complete report (1911²) was given in a paper entitled "Etiology of Canine Distemper." Almost simultaneously with the second report an article (1911³) appeared, by M'Gowan, of Edinburgh, in which was described the same organism. This year an article (1913⁴) appeared, by Torrey and Rahe, of New York, corroborating the findings of the two previous workers.

It is important to note that the organisms found by these investigators were identical, and that their work was carried on absolutely independently of each other, thus establishing in a very conclusive and decisive manner the etiology of the disease.

Soon after the publication of the article by M'Gowan, an opportunity was afforded the writer to study a similar condition, in epizootic form, among rabbits, guinea-pigs, ferrets and monkeys. He was able to corroborate the results of M'Gowan, and demonstrate the identity of the causal organism with the one described as the cause of distemper in dogs, and also prove the relationship and specific nature of these infections (1912⁵, 1912⁶, 1913⁷).

The diseases encountered in epizootic form, which constitute the subject of this paper, include those mentioned in previous papers (1911², 1912⁵, 1912⁶), as well as an infection among rabbits due to an organism of the rabbit septicæmia type, and in infection among dogs due to an organism of the colon type.

Although the organism, first named by the writer *B. bronchicanis* (1911²), and later changed by him to *B. bronchisepticus* (1912⁵), was isolated in the large majority of cases from the trachea, bronchi, nasal cavity, lungs, blood, spleen, liver, intestinal tract and other locations, other micro-organisms, rather closely allied, culturally, have been found, in some cases, in large

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enough numbers to claim attention. Some of these organisms were found contaminating the *B. bronchisepticus*, and others were in pure culture. Those found associated with the *B. bronchisepticus* were undoubtedly secondary invaders, as were also those found alone. In the latter instance, however, the primary infecting agent, the *B. bronchisepticus*, were either overgrown or had disappeared, and the animals had yielded to the secondary infecting agent, the *B. bronchisepticus*, was either overgrown and gives this as an explanation as to why the *B. bronchisepticus* is not always found in the later stages of the disease. The writer showed very conclusively in his earlier articles on the subject, that, in order to obtain the specific organism without much difficulty, the case must be taken in the early stages of the disease. M'Gowan (1911⁸) also, in an epidemic among cats due to this same organism, found that the animals died from secondary infections, which, in some cases, had entirely supplanted the primary.

One object of this paper is to describe the various organisms resembling the *B. bronchisepticus*, and some experiments carried out with them, to determine, if possible, the exact status of the *B. bronchisepticus* as regards micro-organisms of similar morphology which are found at times associated with it. This work is the outcome of a discussion of a paper (1913⁷) on the subject of distemper, in which the question was raised as to whether the *B. bronchisepticus* was one of a large group of organisms with slight cultural variations, or a distinct species.

Before considering the experiments in detail, however, it may be well to give, briefly, the symptomatology and bacteriology of the infections, as found by the writer, in laboratory animals.

INFECTIONS WITH *B. BRONCHISEPTICUS*.

DOG.—*Symptomatology*.—Cough, diarrhoea, serous, followed by purulent discharges from the nose and eyes, together with loss of appetite and flesh, are the most pronounced symptoms. The disease may be ushered in with convulsions, especially in the very young, and result in death within a few hours. A pustular skin lesion is found in about 10 to 15 per cent. of cases. Acute symptoms usually last from a few days to two or three weeks, and are followed by death in 60 to 90 per cent. of cases. The case may go on to a complete recovery or to a chronic condition

lasting several months, or it may terminate in a condition called "chorea." Incubation period is from five to seven days.

Bacteriology.—In the very early stages the *B. bronchisepticus* may be found in pure culture in the respiratory tract, blood, and often in the abdominal organs, and it may be isolated from the intestinal tract. In later stages the specific micro-organism is found associated with a variety of secondary organisms, especially the *Staphylococcus albus* and *Streptococcus pyogenes*.

GUINEA-PIG.—*Symptomatology.*—The symptoms first noticed are weakness, diarrhœa, loss of appetite and flesh. When observed in the cage at rest or walking about, the back will be arched and the hindquarters drawn up; this attitude is very characteristic. Death will follow within a few days in a large majority of pigs affected. Females are more susceptible than males, and the prognosis is invariably fatal in pregnancy. Incubation period is from three to five days.

Bacteriology.—The specific micro-organism is found in the upper respiratory tract in pure culture, and often in the abdominal organs and intestinal canal in association with other organisms. The animal usually dies before secondary organisms become very much in evidence, except in the intestinal canal; wherein it differs from the dog.

RABBIT.—*Symptomatology.*—Loss of appetite and flesh with decreased activity are usually the initial symptoms. Diarrhœa is an invariable symptom, while a discharge from the nose and eyes (purulent very early) is recognized in most cases; wherein it differs from the guinea-pig. Death in the majority of cases is found in from two to ten days, although the disease is not so fatal as with the guinea-pig. Incubation period from five to seven days.

Bacteriology.—In the early stages the *B. bronchisepticus* is usually found in the respiratory tract in pure culture. It may also be found in the blood and abdominal organs. In later stages the micro-organism is associated with pyogenic organisms of secondary infections.

MONKEY.—*Symptomatology.*—Loss of appetite and flesh with decreased activity are about the first symptoms noticed. Diarrhœa is invariably present and an occasional cough is heard. A discharge from the nose or eyes has not been observed. The disease usually lasts from one to two weeks. The incubation period is about one week.

Bacteriology.—The *B. bronchisepticus* has been isolated in pure culture from the respiratory tract and blood. It has also been found in the abdominal organs and intestinal canal. Some of the cases terminated in a streptococcus septicæmia.

FERRET.—*Symptomatology*.—The symptoms are very similar to those found in the rabbits, including loss of appetite and flesh, diarrhoea and purulent discharge from nose and eyes.

Bacteriology.—*B. bronchisepticus* found in respiratory tract in pure culture in early stages. In later stages it is liable to be overrun with the secondary invaders.

RABBIT SEPTICÆMIA.

Symptomatology.—The duration of the disease is, as a rule, but a few hours, so that the symptomatology is not very important. Often a slight discharge may be found at the nostrils, but this is not so profuse nor so purulent as is found in the "snuffles" due to *B. bronchisepticus*. A slight discharge, in fact, seems to be a common symptom of most infections in the rabbit. An infection with the bacillus of rabbit septicæmia seems to be about the most fatal of any of the rabbit diseases.

Bacteriology.—A general invasion of the body with the specific micro-organism.

INFECTION OF DOGS WITH AN ORGANISM OF THE COLON-PARACOLON TYPE.

Symptomatology.—Practically the only symptoms noticeable were a rapid emaciation and diarrhoea, coincident with a loss of appetite resulting in death within a few days.

Bacteriology.—The micro-organism, thought to be the *B. enteritidis*, which was found to be the cause, was isolated in pure culture from the blood and organs. Cultures taken from the trachea and lungs gave no growth. The intestinal canal was not cultured.

DESCRIPTION OF THE SECONDARY ORGANISMS UNDER DISCUSSION WITH THE *B. BRONCHISEPTICUS* FOR COMPARISON.

Only those motile, Gram-negative bacilli which resembled the *B. bronchisepticus* by their early growth on agar were retained for study. All cultures were under observation two weeks when the final readings were taken. No attempt has been made to identify these organisms other than to place the organisms of Group I. with the *B. enteritidis* group.

B. BRONCHISEPTICUS.

Morphology.—Short, narrow. *Agar stroke*.—Moderate, filiform, moist, glistening, translucent. *Bouillon*. Cloudy with sediment. *Potato*. Abundant, dark tan. *Litmus milk*.—Marked alkaline. *Glucose agar*.—No gas. *Gelatin*.—No liquefaction.

GROUP I. (B. ENTERITIDIS.)

No. of strains isolated—17. *Morphology*.—Medium size, broad. *Agar stroke*.—Moderate, lobate, glistening, translucent. *Bouillon*.—Cloudy at first, tendency to clear up later. *Potato*.—Slight, colorless. *Litmus milk*.—Acid, changing to alkaline. *Glucose agar*.—Gas. *Gelatin*.—No liquefaction.

GROUP II.

No. of strains—2. *Morphology*.—Long, narrow. *Agar stroke*.—Moderate, lobate, glistening. *Bouillon*.—Cloudy first, clears up with sediment. *Potato*.—Abundant, cream. *Litmus milk*.—Marked acid, no coagulation. *Glucose agar*.—Gas. *Gelatin*.—No liquefaction.

GROUP III.

No. of strains—1. *Morphology*.—Long, narrow, bipolar. *Agar stroke*.—Abundant, spreading, glistening, turning the medium darker. *Bouillon*.—Flocculent at first, clears with heavy film and heavy sediment. *Potato*.—Slight, colorless. *Litmus milk*.—Color disappears at first, later acid. *Glucose agar*.—No gas. *Gelatin stab*.—Liquefaction.

GROUP IV.

No. of strains—1. *Morphology*.—Small. *Agar stroke*.—Moderate, filiform. *Bouillon*.—Clear and viscid sediment. *Potato*.—Moderate, tan. *Litmus milk*.—Slightly alkaline. *Glucose agar*.—No gas. *Gelatin*.—No liquefaction.

GROUP V.

No. of strains—1. *Morphology*.—Large. *Agar stroke*.—Moderate, echinulate. *Bouillon*.—Cloudy. *Potato*.—Slight, colorless. *Litmus milk*.—Acid, coagulation. *Glucose agar*.—Gas. *Gelatin*.—Liquefaction.

GROUP VI.

No. of strains—3. *Morphology*.—Large. *Agar stroke*.—Moderate, lobate. *Bouillon*.—Cloudy. *Potato*.—Abundant, yellow. *Litmus milk*.—Acid, coagulation. *Glucose agar*.—Gas. *Gelatin*.—No liquefaction.

GROUP VII.

No. of strains—2. *Morphology*.—Large. *Agar stroke*.—Moderate, lobate. *Bouillon*.—Cloudy. *Potato*.—Abundant, cream. *Litmus milk*.—Slightly acid, changing to alkaline. *Glucose agar*.—No gas. *Gelatin*.—No liquefaction.

While the variations between these organisms were decisive enough to clearly differentiate them, agglutination tests were carried on as controls, to complete the work and corroborate the classifications. It will be seen that in all cases the agglutination tests confirmed absolutely the cultural work.

Immune sera were obtained from rabbits injected with these various organisms, as well as with various strains of *B. bronchisepticus* obtained from different animals, for the purpose of cross agglutinations, as controls. After first obtaining a small quantity of serum from each rabbit, as a normal control, the animals were given three injections of a fairly heavy suspension of dead organisms in physiological salt solution, intravenously, at intervals of three days, and bled one week after the last injection. The suspensions for the agglutination tests were prepared according to a method described in a previous article (1911²). The tests were allowed to stand in the incubator, and readings were taken at the end of twenty-four hours.

TABLE II.

Suspension of <i>B. bronchisepticus</i> from	Agglutination Titre of Sera from Rabbits immunised with <i>B. bronchisepticus</i> from				
	Rabbit.	Monkey.	Guinea-pig.	Dog.	Intestine of Puppy.
Rabbit	1 : 3200	1 : 6400	1 : 1000	1 : 3200	1 : 3200
Monkey	1 : 1600	1 : 6400	1 : 1800	1 : 2000	1 : 1000
Guinea-pig	1 : 1600	1 : 3200	1 : 2000	1 : 2000	1 : 2000
Dog	1 : 1600	1 : 3200	1 : 1600	1 : 3200	1 : 3200
Intestine of puppy . . .	1 : 1600	1 : 3200	1 : 1800	1 : 3200	1 : 3200
Control (normal serum) .	1 : 10	1 : 20	1 : 10	0	0
Ferret	1 : 2000	...
Douglass	1 : 2000	...
Human	1 : 3200	...

EXPERIMENT 1 (Table II).—Test of several strains of *B. bronchisepticus* isolated from various sources. Cross-agglutina-

tions were carried out, except with the strains marked ferret, Douglass, and human.

EXPERIMENT 2.—In this experiment (Table III) suspensions from the organisms resembling *B. bronchisepticus* were tested against a serum immune to strain of *B. bronchisepticus* from dog 36. This serum has always given typical and high agglutination with suspension of *B. bronchisepticus*. It will be seen that the highest titre obtained from any of the secondary organisms was 1:400, from Group III, while that from *B. bronchisepticus* was 1:3200. Comparing this with the previous experiment, it will be seen that agglutinations of *B. bronchisepticus* suspensions with their homologous sera are always above 1:1000 and average 1:3200.

TABLE III.—*Agglutination Titre of Serum from a Rabbit.*

(Immunised with *B. bronchisepticus*, No. 36.)

Suspensions of			
<i>B. bronchisepticus</i>	No. 36	1 : 3200
Group I.	Bacillus	" 14	0
	"	" 26	0
	"	" 34	0
	"	" 35	0
	"	" 36	0
	"	" 45	0
Group II.	"	" 38	0
Group III.	"	" 52	1 : 400
Group IV.	"	" 54	...
Group V.	"	" 143	1 : 100
Group VI.	" 191	1 : 100
		" 209	1 : 80
Group VII.	" 161	0
		" 162	1 : 10

Group I, which was found in a larger number of cases than any of the other secondary organisms (seventeen out of twenty-seven), showed no tendency whatever to agglutinate.

EXPERIMENT 3.—This experiment (Table IV) shows the result of the cross-agglutinations between the organisms of the various groups.

The horizontal rows represent the immune sera. The per-

TABLE IV.

Serum from Rabbit treated with												
<i>B. bronchisepticus</i>	Suspensions	<i>B. bronchisepticus</i>		Group I.		Group II.	Group III.	Group IV.	Group V.	Group VI.	Group VII.	
		No. 36	No. 36	No. 26	No. 140	No. 38	No. 52	No. 54	No. 143	No. 192	No. 161	
<i>B. bronchisepticus</i>	{ Bac. No. 12 " 17 " 25 " 34 " 35 " 36 " 45 " 47 " 63 " 71 " 105 " 140 " 141 " 160 " 177 " 199	{ <i>B. bronchisepticus</i> " 33 " 41 " 52 " 54 " 143 " 191 " 192 " 209	<i>B. bronchisepticus</i>	<i>I. 3200</i>	1:10	0	
			Spontaneous	agglutination
			0	1:3200+	1:40	0	0
			..	1:3200+
			0	1:3200+
			0	1:3200+
			0	1:6400
			0	1:3200+	1:100	1:10	0	0	0	0	0	0
			..	1:3200+
			..	1:3200+
Group I.	{ " 33 " 41 " 52 " 54 " 143 " 191 " 192 " 209	{ <i>B. bronchisepticus</i> " 33 " 41 " 52 " 54 " 143 " 191 " 192 " 209	Spontaneous	agglutination	
			"	"
			"	"
			..	1:3200+
			0	1:80	1:400	1:40	1:100	1:100	1:20	1:400	1:50	
			..	1:80
			1:400	1:100	1:40	<i>I. 10000+</i>	1:3200+	1:100	1:100	1:400	1:400	
			Spontaneous	agglutination
			1:400	1:400	1:200	1:40	1:10	1:1000+	1:400	1:400	1:400	
			1:100	1:200	1:40	1:100	1:20	1:400	1:400	1:400	1:400	
Group VI.	{ " 191 " 192 " 209	{ <i>B. bronchisepticus</i> " 33 " 41 " 52 " 54 " 143 " 191 " 192 " 209	1:80	1:200	
			0	0	1:1000	1:20	1:40	1:100	1:20	1:400	1:400	1:400
			1:10
		
		
		
		
		
		
		
Group VII.	{ " 161 " 162	{ <i>B. bronchisepticus</i> " 33 " 41 " 52 " 54 " 143 " 191 " 192 " 209	0	0	1:20	1:200	1:40	1:100	1:20	1:1000+		
			1:10

pendicular rows represent the suspensions. The figures in italics represent the titre of the suspensions of various bacilli with their homologous serums. The suspensions of *B. bronchisepticus* with their homologous serums gave an agglutination on the average of about 1:3200, while the organisms of the remaining groups appeared to have a tendency to agglutinate in very much higher dilutions, most of them going up as high as 1:10,000. The suspensions of the organism from Group IV. always gave a spontaneous reaction, so that the only means we had of testing this organism was by means of its immune serum against suspension of organisms from the other groups.

CONTROL OF EPIZOOTICS AMONG LABORATORY ANIMALS.

If infectious diseases among laboratory animals are to be controlled and epizootics prevented, especially where large numbers are housed together, as in breeding stables, strict hygienic measures must be adhered to and sanitary precautions carried out. This includes, wherever applicable, preventive inoculations with specific vaccines.

As regards infections caused by *B. bronchisepticus*, experience has taught us that epizootics can be controlled and a protection afforded the susceptible animals, provided prophylactic injections with vaccines, together with the ordinary sanitary measures, are intelligently and systematically carried out. In the epizootics under discussion, among guinea-pigs and rabbits, due to *B. bronchisepticus*, vaccines were made up with the specific bacillus, 100,000,000 per cubic centimetre. Each animal was injected with its homologous vaccine every third day, starting with 1 c.c. and increasing the dose 1 c.c. at each subsequent injection.

The epizootic, due to the colon-like bacillus, which simulated an acute type of distemper, and might easily have been mistaken for it, was found among a number of young dogs which were being saved for experimental purposes, and had therefore received their regular prophylactic vaccinations against *B. bronchisepticus*. It was thought at first that the dogs were suffering from true distemper, and that the vaccine in this case was not protecting. After a thorough bacteriological examination had been made of every dog that died, it was found that the *B. bronchisepticus* was not present and that another bacillus of a

very similar morphology was responsible for the trouble. This bacillus, which was probably the *B. enteritidis*, was found to be extremely virulent, a very small dose, upon subcutaneous inoculation, killing a dog within a day or two with an acute general infection.

This incident showed very strikingly that at least one disease similar to the acute form of distemper may at times appear in epizootic form; and in order to differentiate the condition from distemper a bacteriological examination would be necessary.

The epizootic, due to the bacillus of rabbit septicæmia type, did not respond so readily, in our hands, to prophylactic injection with a specific vaccine. A vaccine made up with this bacillus, 100,000,000 per c.c., given in three injections of increasing doses, offered practically no protection. It was found, experimentally, also, that three doses did not protect rabbits from the M. F. D. of this organism. The results of the experimental as well as the clinical tests convinced us, therefore, that, if we are to protect against the disease, at least a more protracted course of treatment with the prophylactic vaccine would be necessary. This disease was finally controlled only by isolation.

CONCLUSIONS.

From the results of the cultural tests and agglutination experiments, as carried out on the micro-organisms included in this study, it is shown very clearly that the *B. bronchisepticus* is a distinct species. This confirms the previous work of M'Gowan and also the writer, and corroborates the results of Torrey, who says: "We have tested many strains of the bacillus, and in every instance in which there was an exact correspondence with the diagnostic cultural tests there was also a definite agglutination with a single anti-serum." "As regards agglutination, then, there are no sub-varieties of the bacillus, but the same degree of uniformity obtains which is encountered with *Vibrio cholera* or *B. typhosus*."

It has been found by Torrey, M'Gowan, and the writer, after studying many strains of the bacillus, that the cultural tests are invariable, and, for ordinary routine work, the reactions on litmus milk and potato are characteristic, and that the bacillus

may be identified by its behavior towards these media. The writer has encountered but two organisms which may stimulate the *B. bronchisepticus* in its reactions towards these media; namely, the *B. fecalis alkaligenes*, mentioned also by Torrey, and an organism described in this paper as bacillus of Group IV. The *B. fecalis alkaligenes*, however, according to different authorities, does not always produce a characteristic alkaline reaction in litmus milk, while its growth on potato is but slightly raised and not so moist. A culture of this organism received from the American Museum of Natural History, New York, gave, in the hands of the writer, a distinct acid reaction in litmus milk. The organism described in this paper as bacillus of Group IV might, according to its growth on potato and in litmus milk, be identified as *B. bronchisepticus*, were it not for its growth in bouillon. The bouillon was clear with a stringy sediment, instead of being cloudy. This characteristic clumping was shown very clearly in the agglutination work with this organism. The suspensions of this organism invariably agglutinated spontaneously.

REFERENCES.

1. FERRY: "A Preliminary Report of the Bacterial Findings in Canine Distemper," *Amer. Veter. Rev.*, 1910, vol. xxxvii. p. 499.
2. FERRY: "Etiology of Canine Distemper," *Journ. Infect. Diseases*, Chicago, 1911, vol. viii. p. 399.
3. M'GOWAN: "Some Observations on a Laboratory Epidemic, principally among Dogs and Cats, in which the Animals affected presented the Symptoms of the Disease called Distemper," *Journ. Path. and Bacteriol.*, Cambridge, 1911, vol. xv. p. 372.
4. TORREY: "Studies in Canine Distemper," *Journ. Med. Research*, Boston, 1913, vol. xxvii. p. 291.
5. FERRY: "Further Studies on the Bacillus Bronchicanis, the Cause of Canine Distemper," *Amer. Veter. Review*, 1912, vol. xli, p. 77.
6. FERRY: "*Bacillus bronchisepticus* (Bronchicanis): The Cause of Distemper in Dogs and a Similar Disease in other Animals," *The Veterinary Journal*, 1912, vol. lxviii. p. 376.
7. FERRY: "*Bacillus bronchisepticus*: Its Relation to Canine Distemper," *Amer. Veter. Review*, 1912, vol. xliii, p. 16.
8. M'GOWAN: "On an Epidemic among Cats, supervening on and simulating Distemper," *Journ. Path. and Bacteriol.*, Cambridge, 1911, vol. xvi. p. 257.
9. M'GOWAN: "Some Observations on the Clinical Symptoms, Prophylaxis and Treatment of Distemper," *The Veter. Journ.*, 1912, vol. lxviii. p. 7.

**REPRINTS OF PUBLICATIONS FROM THE RESEARCH
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The present system of collecting reprints of articles published from the Research Laboratory was begun in 1912. Reprints of the following articles published subsequent to that time are available and will be sent upon request.

1. On the Administration of Diphtheria Toxin in a Collodion Sac. By E. C. L. Miller. (*Journal of Infectious Diseases*, Vol. 8, January, 1911, pp. 50-65.)

2. A Further Contribution to Our Knowledge of Insecticides—Fumigants. By Chas. T. McClintock, H. C. Hamilton and F. B. Lowe. (*Journal of the American Public Health Association*, Vol. 1, April, 1911, pp. 227-238.)

3. *Duboisia Hopwoodii*—A Histological Study. By Oliver A. Farwell. (Reprinted from *Merck's Report*, Vol. 20, May 1, 1911.)

4. Etiology of Canine Distemper. By Newell S. Ferry. (*Journal of Infectious Diseases*, Vol. 8, June, 1911, pp. 399-420.)

5. The Resistance of Smallpox Vaccine to the Coal-tar Disinfectants. By Chas. T. McClintock and Newell S. Ferry. (*Journal of the American Public Health Association*, Vol. 1, June, 1911, pp. 418-419.)

6. Production of Immunity with Over-Neutralized Diphtheria Toxin. By Chas. T. McClintock and Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Originale, Bd. 59, July 15, 1911, pp. 456-464.)

7. Soaps from Different Glycerides—Their Germicidal and Insecticidal Values Alone and Associated with Active Agents. By H. C. Hamilton. (*Journal of Industrial and Engineering Chemistry*, Vol. 3, August, 1911, pp. 582-584.)

8. The Sleepy Grass of New Mexico: A Histological Study. By Oliver A. Farwell. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-273.)

9. Some Observations on the Physiological Action of Sleepy Grass. By A. W. Lescohier. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-275.)

10. An Investigation of the Depressor Action of Pituitary Extracts. By Carey P. McCord. (*Archives of Internal Medicine*, Vol. 8, November, 1911, pp. 609-620.)

11. The Physiology of the Pituitary Gland and the Action of Its Extracts. By Carl J. Wiggers. (*American Journal of Medical Sciences*, Vol. 141, April, 1911, pp. 502-515.)

12. A Physiological Investigation of the Treatment of Hemoptysis. By Carl J. Wiggers. (*Archives of Internal Medicine*, Vol. 8, 1911, pp. 17-38.)

13. Notes on Catgut Sterilization: A Preliminary Report. By Wil-
lard H. Hutchings. (*Annals of Surgery*, Vol. 54, July, 1911, pp. 693-695.)

14. The Relations of Pyogenic Microorganisms to the Etiology and Treatment of Skin Diseases. By Henry Rockwell Varney. (*Ohio State Medical Journal*, December, 1911.)

15. A Micrococcus with Unusual Characteristics as a Factor in a Resistant Dermatitis Resembling Acne Vulgaris. By Henry Rockwell Varney and L. T. Clark. (*Journal of Cutaneous Diseases*, Vol. 30, February, 1912, pp. 72-78.)

16. Serum Treatment of Hemorrhage and Blood Dyscrasias. By A. W. Lescohier. (*New York Medical Journal*, Vol. 95, February 3, 1912, pp. 223-229.)

17. Further Studies on the Bacillus Bronchicanis, the Cause of Canine Distemper. By Newell S. Ferry. (*American Veterinary Review*, Vol. 41, April, 1912, pp. 77-79.)

18. The Pharmacopoeial Requirements for Cannabis Sativa. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, March, 1912, pp. 200-203.)

19. The Heart Tonic Unit. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 84, March, 1912, pp. 97-103.)

20. Studies on the Etiology of Equine Influenza. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, April, 1912, pp. 185-197.)

21. A Method for the Bacteriological Standardization of Disinfectants. By Tatsuzo Ohno and H. C. Hamilton. (*American Journal of Public Health*, Vol. 2, May, 1912, pp. 331-338.)

22. Physiological Testing. By E. M. Houghton. (*American Drug-gist*, July and September, 1911, and January and April, 1912.)

23. Bacillus Bronchisepticus (Bronchicanis): The Cause of Distemper in Dogs and a Similar Disease in Other Animals. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, July, 1912, pp. 376-391.)

24. On Feeding Young Pups the Anterior Lobe of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 30, July, 1912, pp. 352-357.)

25. A Practical Portable Incubator. By Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Original, Bd. 65, Heft 4/5, 1912, pp. 412-413.)

26. Tobacco Extracts: Their Comparative Values as Insecticides. By W. O. Hollister. (*Journal of Economic Entomology*, Vol. 5, June, 1912, pp. 263-267.)

27. The Pharmacological Assay of Pituitary Preparations. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, October, 1912, pp. 1117-1119.)

28. Pituitary Extracts in Obstetrics and Gynecology. By A. W. Lescohier and O. E. Closson. (*Journal of the Michigan State Medical Society*, Vol. 11, October, 1912, pp. 650-657.)

29. Biological Products—Veterinary. By Robert H. Wilson. (*American Veterinary Review*, Vol. 41, September, 1912, pp. 668-681.)

30. The Isolation and Cultural Characteristics of Bacillus Acne. By Edwin M. Stanton. (*Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Original, Bd. 66, Heft 5/7, 1912, pp. 386-389.)

31. Studies on Hog Cholera. By Walter E. King and Robert H. Wilson. (*Journal of Infectious Diseases*, Vol. 11, Nov., 1912, pp. 441-458.)

32. Studies on the Virus of Hog Cholera. By Walter E. King and F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 39-41.)

33. The Physiological Activity of Cannabis Sativa. By H. C. Hamilton, A. W. Lescohier and R. A. Perkins. (*Journal of the American Pharmaceutical Association*, Vol. 2, Jan., 1913, pp. 22-30.)

34. The Iodine Content of the Small, Medium and Large Thyroid Glands of Sheep, Beef and Hogs. By T. B. Aldrich. (Original Communications, Eighth International Congress of Applied Chemistry, Vol. XIX, 1912, pp. 9-14.)

35. Studies on the Virus of Hog Cholera. By Walter E. King and Robert H. Wilson. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 16, Heft 3, 1913, pp. 367-376.)

36. On the Cultivation of the Treponema Pallidum (Spirochæta Pallida). By F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 55-67.)

37. Studies on the Gonococcus. I. By Carl C. Warden. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 93-105.)

38. Studies on the Virus of Hog Cholera. By Walter E. King, F. W. Baeslack and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 12, March, 1913, pp. 202-205.)

39. Bacillus Bronchisepticus—Its Relation to Canine Distemper. By N. S. Ferry. (*American Veterinary Review*, Vol. 43, April, 1913, pp. 16-30.)

40. Drug Influence on Extrasystoles of the Mammalian Heart. By Carey P. McCord. (*Interstate Medical Journal*, Vol. 19, Oct., 1912, pp. 870-880.)

41. The Employment of Protective Enzymes of the Blood as a Means of Extracorporeal Diagnosis. I.—Sero-Diagnosis of Pregnancy. By Carey P. McCord. (*Surgery, Gynecology and Obstetrics*, Vol. 16, April, 1913, pp. 418-421.)

42. Tribromo-tert-Butyl Alcohol, $C_4H_9OBr_3$. By T. B. Aldrich. (*Journal of the American Chemical Society*, Vol. 33, March, 1911, pp. 386-388.)

43. On Feeding Young White Rats the Posterior and the Anterior Parts of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 31, Nov., 1912, pp. 94-101.)

44. The Rationale of the Use of Adrenalin in the Treatment of Asthma. By Carey P. McCord. (*Medical Record*, Vol. 83, March 8, 1913, pp. 431-432.)

45. Standardization of Disinfectants: Some Suggested Modifications. By H. C. Hamilton and T. Ohno. (*American Journal of Public Health*, Vol. 3, June, 1913, pp. 582-588.)

46. Preventive Measures Against Equine Influenza Based on Its Bacteriology. By N. S. Ferry. (Report of the Proceedings of the United States Live Stock Association, December, 1912, p. 127.)

47. Correcting Water. By H. C. Hamilton. (*Bulletin of Pharmacy*, Vol. 27, August, 1913, pp. 330-335.)

48. Duration of Immunity Following Small-pox Vaccination. By A. W. Lescohier. (*Journal of the American Medical Association*, Vol. 66, Aug. 16, 1913, page 487-490.)

49. On Crystalline Kombe-Strophanthin. By D. H. Brauns and O. E. Closson. (*Journal of the American Pharmaceutical Association*, May, June and July, 1913, Vol. 2.)

50. A Comparative Study of Antigens for the Wassermann Reaction. By H. R. Varney and F. W. Baeslack. (*Journal of the American Medical Association*, Vol. 66, Sept. 6, 1913, pp. 754-757.)

51. The Treatment of Tetanus. By Charles T. McClintock and Willard H. Hutchings. (*Journal of Infectious Diseases*, Vol. 13, Sept., 1913, pp. 309-320.)

52. Spirochæta Suis, Its Significance as a Pathogenic Organism, Studies on Hog Chlorea. By Walter E. King and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 13, Nov., 1913, pp. 463-498.)

53. Time Recorder for Kymograph Tracings. By Oliver E. Closson (*Journal of Pharmacology and Experimental Medicine*, Vol. 5, Jan., 1914, pp. 235-238.)

54. U. S. P. Menstrua. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 86, Feb., 1914, pp. 56-61.)

55. Numerical Variations of the White Blood Cells in Mice Inoculated with Transplantable Adenocarcinoma. By F. W. Baeslack. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 20, Heft 5, 1914, pp. 421-435.)

56. A Study of the Germicidal Action of the Ultraviolet Rays. By E. M. Houghton and L. Davis. (*American Journal of Public Health*, Vol. 4, March, 1914, pp. 224-240.)

57. Some Phenomena Involved in the Life History of *Spirochæta Suis*—Studies on Hog Cholera. By W. E. King and R. H. Drake. (*The Journal of Infectious Diseases*, Vol. 14, March, 1914, pp. 246-250.)

58. The Sterilization of Adrenalin Solutions. By L. W. Rowe. (*American Journal of Pharmacy*, Vol. 86, April, 1914, pp. 145-149.)

59. Infection and Immunity: A Review. By N. S. Ferry, Ph.B., M.D. (*Journal of the American Pharmaceutical Association*, Vol. 3, April and May, 1914.)

60. Disinfection—What Disinfectant is the Most Generally Applicable for Clinical, Surgical and Sanitary Purposes? By H. C. Hamilton. (*Therapeutic Gazette*, Vol. 38, May, 1914, pp. 311-315.)

61. Study of the Bacteriology of the Posterior Nasopharynx in Scarlatina. By N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, pp. 934-935.)

62. Some Experiences with Bacterial Vaccines in Scarlatina. By Guy L. Kiefer, M.D., D.P.H., and N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, p. 936.)

63. A Sero-enzyme Test for Syphilis. By F. W. Baeslack, M.A., M.D. (*The Urologic and Cutaneous Review*, Vol. 18, May, 1914, pp. 234-238.)

64. Bacteriology and Control of Acute Infections in Laboratory Animals. By N. S. Ferry, Ph.B., M.D. (*Journal of Pathology and Bacteriology*, Vol. 18, May, 1914, p. 445.)

THE BACTERIOLOGICAL STANDARDIZATION OF DISINFECTANTS.

SOME FURTHER SUGGESTIONS.

H. C. HAMILTON AND TATSUZO OHNO.

(From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.)

Read before the Laboratory Section, American Public Health Association, Colorado Springs, September, 1913.

When any substance which finds application directly or indirectly in therapeutic measures cannot readily be standardized by any chemical method, other methods are usually attempted by which to determine its value. The medicinal substances which fail to respond to any chemical assay method are standardized by various means, of which perhaps the most important is the biological assay.

An example of valuable products whose values cannot, in most cases, be determined by chemical assay is that of the coal-tar disinfectants. While it is established that the value of the coal-tar oils as disinfectants resides largely if not entirely in those constituents similar to phenol, the crude product contains these phenols in such an endless variety that the chemical assay of any oil can give only a hint as to the actual value of that oil as a germicide. We resort, therefore, to a bacteriologic method and attempt to decide its value by testing it as a germicide.

It is almost impossible to make a laboratory test of a disinfectant which will duplicate the practical use of that product even in one particular case. How much more difficult it would be to duplicate every use to which such a product may be applied, is very evident. The infinite variety of conditions under which disinfection is practised opens wide the field for discussion as to the minor points which it is desirable to consider in outlining a method for their standardization.

This is but natural. One investigator, realizing the difference in resistance of different organisms, chooses an exceptionally resistant one, as *B. pyocyaneus*. Another, who is vitally interested in the disinfection of excreta, suggests *B. typhosus*. Another, wishing to avoid the danger lurking in a culture of *B. typhosus*, chooses *B. coli communis*.

Again, one investigator considers that a disinfectant should be valued on its prompt action and suggests that the dilution to be compared with standard should be that which kills the organism in a time between one and five minutes. Another suggests a half-hour as the maximum time, on the logical supposition that the disinfectant will be acting for at least that period. So, too, opinions vary as to the temperature at which disinfectants should be tested and as to the medium in which the organism should be grown. While these points are not unimportant, any method which attempts to incorporate all of the possible suggestions would necessarily be too cumbersome for practical use.

Looking over the field of discussion, it is evident that one may eliminate as less important all methods but two; namely, that proposed by Doctor Samuel Rideal and T. Ainslie Walker and that proposed by Doctors John F. Anderson and Thomas B. McClintic, known, respectively, as the Rideal-Walker and Hygienic Laboratory Methods. The others may be eliminated from any specific reference because so many of their valuable features have been incorporated in these two methods.

The authors of this paper published a method (*American Journal of Public Health*, May, 1912) which has been in practical use for the standardization of commercial disinfectants for a period of fourteen years. In its essential details, the Rideal-Walker Method resembles it so closely that for all practical purposes they are the same.

In a later paper (*American Journal of Public Health*, June, 1913) the authors suggested some changes in following the Hygienic Laboratory Method, changes which appear to simplify what is really a complicated process. In that paper reference was made to the possibility of evolving from the wealth of material at hand a simple practical method for standardizing disinfectants.

At this time we wish to present some data pointing to the marked variation in the results of germicidal assays by the Hygienic Laboratory Method. This data consists of: first, reports of tests of two disinfectants which had been submitted to three bacteriologic laboratories; second, the reported coefficients on several well-known disinfectants by various investigators; third, results obtained by using, as the test organism,

two different strains of *B. typhosus* grown in the same culture medium; fourth, results of a long series of tests on two disinfectants under a variety of conditions, including the use of test organisms *B. typhosus*, Hopkins, obtained from different sources and from the same source at different times; also the growing of the organism on or in different media.

As a conclusion to these illustrations, we wish to suggest certain steps which might be adopted to advantage as a means of obtaining uniform results in standardizing disinfectants.

When the Hygienic Laboratory Method was made public by the appearance of *Bulletin No. 82*, the authors of this paper immediately set about becoming familiar with the technique, with the idea that the method would soon become official. Such variable results were obtained, however, that it was decided to submit two samples to three prominent bacteriologists.

The remarks of these bacteriologists quoted in the letters below are sufficient to indicate that the method is one which leaves much to be desired. From letter dated January 28, 1914, signed by Dr. Herbert D. Pease, we excerpt the following:

"I believe now that it is, or will very soon be, possible to obtain results with the Hygienic Laboratory Phenol Coefficient Method which would be within 10 per cent. or 15 per cent. of each other. What I stated in my letter held good at that time, but I do not think it holds true in all respects at the present time. We are making a critical analysis of our methods at the present time and I expect to have them very greatly improved in the near future.

"Some of the points that I brought out in my letter of February 19 are still expressive of my opinion. The last sentence of the first paragraph, 'The variations in results are always greater the higher the coefficients, and are smaller the lower the coefficients.' I think even the percentage variation is slightly greater with the higher coefficients than with the lower.

"I do not believe we have yet learned all there is to know about the Hygienic Laboratory Method. It is quite possible that we can improve it, although I do not see very clearly at the present time just wherein any changes would produce such a result. I believe we should all keep working at the matter as far as we possibly can—I intend to try to do my part."

Hygienic Laboratory Phenol Coefficient No. 578273 $\left\{ \begin{array}{l} \frac{27.5}{8.0} = 3.43 \\ \frac{40.0}{11.0} = 3.63 \end{array} \right\} = 3.53.$

Hygienic Laboratory Phenol Coefficient No. 581507 $\left\{ \begin{array}{l} \frac{27.5}{8.0} = 3.43 \\ \frac{37.5}{10.0} = 3.57 \end{array} \right\} = 3.59$

(signed) H. D. PEASE

The letter of Dr. Joseph McFarland we quote in full as follows:

PHILADELPHIA, January 14, 1913.

We have at last completed the tests of Kreso 1 (R 578273) and Kreso 2 (R 581507). It was a long and tedious piece of work, fraught with many difficulties, and complicated by the rapidity of transplantation necessitated by the method worked out by Anderson.

The culture employed for the tests was the "Hopkins Typhoid Bacillus" obtained from Dr. Anderson. The carbolic acid used was Merck's "Absolute." We at first worked with a stock solution made by weighing out both the carbolic acid and the water, but subsequently abandoned this for a new stock solution made by measuring the water and weighing the carbolic acid. As you may note by an examination of the protocols, it is the latter solution that coincides with the solution used by Anderson.

You will see that the results obtained at the different tests were not uniform. To secure, as nearly as possible, the precise strength of the Kreso solution, we made many tests of both and averaged them. We think that their averages give the strength as nearly as it can be determined, Kreso—1=4.58, Kreso—2=5.18.

We hope that these results are in agreement with your own.

Very truly yours,

(Signed) JOSEPH MCFARLAND

Dr. Webster's letter, including report, is as follows:

We enclose herewith report for examinations of Kreso Nos. 581507 and 578273. The phenol coefficient of this disinfectant was determined according to the method of *Bulletin No. 82*, Hygienic Laboratory, Public Health and Marine Hospital Service of the United States.

The delay in getting the report to you was occasioned by the fact that it was necessary to run three different series of tests before we could be sure of the test typhoid organism.

Trusting that this report may be satisfactory to you and thanking you for your favor, we remain,

Very truly yours,

CHICAGO LABORATORY

(Signed) R. W. WEBSTER, per L.

Kreso No 578273 $\frac{100 \times 100}{8.9 \times 11.0} = \frac{5 \times 4.54}{2} = 4.77.$

Kreso No 581507 $\frac{100 \times 100}{8.9 \times 11.0} = \frac{3.75 \times 3.75}{2} = 3.75.$

TABLE I.
REPORTS SUMMARIZED.

Tested by.	Disinfectant.		Date of Tests.
	578273.	581507.	
	Coefficient.		
Dr. Herbert D. Pease.....	3.53	3.59	Oct., 1912.
Dr. Joseph McFarland.....	4.58	5.18	
Dr. R. W. Webster.....	4.77	3.75	
Tatsuzo Ohno.....	5.45	4.95	
H. C. Hamilton.....	4.75	4.3	
Average of the five results.....	4.61	4.35	
Results on different dates.			
Ohno.....	7.1	7.9	July
	4.88	4.53	Oct.
	5.67	5.	Oct.
Hamilton.....	6.	5.	June
	4.75	3.6	Oct.
	5.	4.1	Oct.

That the Hygienic Laboratory Method often gives different results in the hands of different workers, is also evident from the following results compiled from different sources:

TABLE II.

Disinfectant.	Authority.	Result.
F.....	Hamilton	3.9
	Ohno	4.
	Hygienic Laboratory Bull. 82	6.06
	Label	6.
G.....	Hamilton	9.2
	Ohno	9.4
	Hygienic Laboratory Bull. 82	15.
	Label	15-16
H.....	Hamilton	9.
	Ohno	10.
	Pearson	22½
	Hygienic Laboratory	16.6
	* Texas State Board of Health	18.
	Dr. Prescott	12.2
	Walker	22.

*(*American Medicine*, May, 1912)

It is possible that the character of the emulsifying agent in this case (gelatine) may have influenced the results obtained.

These tests were made about a week to ten days after receiving a fresh agar culture of the test organism from the Hygienic Laboratory.

The Hygienic Laboratory Method has invariably given a lower value to a coal-tar disinfectant than one obtained by the other methods cited. The question, therefore, arose, what feature of the tests is responsible for this lower value? By growing culture No. 0190 in the medium used in the Hygienic Laboratory Method, it was at once found that the test organisms are decidedly different in resistance, the Hopkins strain being considerably stronger toward the coal-tar disinfectants than the strain previously employed (No. 0190. See Table IV). The Hopkins strain, however, as is shown in Table I, had at one time not much greater resistance than culture No. 0190.

TABLE IV.
HYGIENIC LABORATORY METHOD.
Hygienic Laboratory Medium.

Min.	Phenol.				D.				E.			
					Hopkins' Culture.							
2½	—	+			—	+			—	+		
15	90	100	110	120	14	15	18	19	8	9	11	12*
			—	+			—	+			—	+
	Coefficient A 15.9								B 9.5			
	Culture No. 0190.											
2½	—	+			—	+			—	+		
15	100	110	120	130	16	17	22	23	10	11	13	14*
			—	+			—	+			—	+
	Coefficient A 17.2								B 104.			

*Dilutions of D and E are in hundreds.

To determine whether the method of growing the organism has any appreciable effect on its resistance, a long series of tests was devised and carried out by the authors working independently. The experiment included the test of three disinfectants

on the Hopkins organism, three different cultures, lettered a, b, and c, being obtained at different times and grown in three ways, namely:

- x continuously on bouillon,
- y " " agar
- z alternately on agar and bouillon.

The first (x) was transplanted daily from bouillon to bouillon. The second (y) was transplanted weekly from agar to agar, a bouillon culture being made every other week and transplanted to bouillon daily. The third (z) was transplanted from bouillon to agar, where it grew one week, then transplanted to bouillon from this medium, transplants being made daily for one week, then to agar again for one week.

The results of seventeen tests covering a period of eight and one-half months is summarized in the following tables:

TABLE V.

Average for each culture grown under each of the three different conditions:

B.		C.	
ax	4.56	ax	9.38
ay	3.45	ay	9.30
az	4.24	az	8.91
bx	4.37	bx	9.12
by	4.28	by	9.43
bz	4.28	bz	9.24
cx	4.75	cx	9.24
cy	3.82	cy	8.81
cz	4.21	cz	9.03

Averages when culture is grown and transplanted differently:

x	4.58	x	9.25
y	4.11	y	9.18
z	4.24	z	9.4

Averages with the different cultures:

a	4.38	a	9.20
b	4.31	b	9.25
c	4.26	c	9.03

The averages shown in these tables are remarkably close, considering what variable results this method of testing has given on other occasions. But when one observes the extremes, the variable results obtained are more apparent.

The following tables show the lowest and highest coefficients obtained with each culture and each way of growing the culture, and shows also the difference between the extremes and the percentage this difference is, of the lowest coefficient.

TABLE VI.
DISINFECTANT B—EXTREME COEFFICIENTS.

	Lowest.	Highest.	Difference.	Per Cent.
ax.....	4.	5.5	1.5	37½
ay.....	3.68	4.9	1.22	33
az.....	3.48	4.92	1.44	41
bx.....	3.88	5.5	1.62	42
by.....	3.8	4.72	.92	24
bz.....	3.48	4.77	1.29	37
cx.....	4.1	5.7	1.6	40
cy.....	3.	4.77	1.77	59
cz.....	3.6	5.	1.4	41

DISINFECTANT C—EXTREME COEFFICIENTS.

	Lowest.	Highest.	Difference.	Per Cent.
ax.....	7.5	10.45	2.95	40
ay.....	7.6	10.4	2.8	37
az.....	7.6	9.54	1.9	25
bx.....	7.7	10.	2.3	30
by.....	7.4	10.7	3.3	44
bz.....	7.5	10.4	2.9	40
cx.....	7.6	10.3	2.7	35
cy.....	7.8	9.5	1.7	22
cz.....	7.7	10.	2.3	30

The plates illustrating this experiment consist of the curves obtained by plotting the averages of the results. The abscissas are the numbers of tests, the ordinates are the dilutions of the disinfectants.

Solid lines are results with cultures grown in bouillon continuously (x). Broken lines are results when the cultures were grown on agar continuously (y). Dotted lines, when the cultures were grown alternately on bouillon and agar (z). Cultures y and z are identical in first test; y, only, is recorded.

A, B, and C are the three disinfectants, A being phenol; a, b, c, the three different cultures used. The black lines show results obtained by Hamilton, the red, those by Ohno.

The location of the points determining the curve was obtained by using the average of the highest dilutions killing at 2½ and 15 minutes. For example, in Plate 1, the first test of carbolic

acid (A), with the culture first obtained from Washington (a), and grown continuously in bouillon (x) by Hamilton (black solid lines), gave an average dilution 115, this being half the sum of the two efficient dilutions, *i.e.*, that allowing no growth in $2\frac{1}{2}$ minutes and that allowing no growth in 15 minutes.

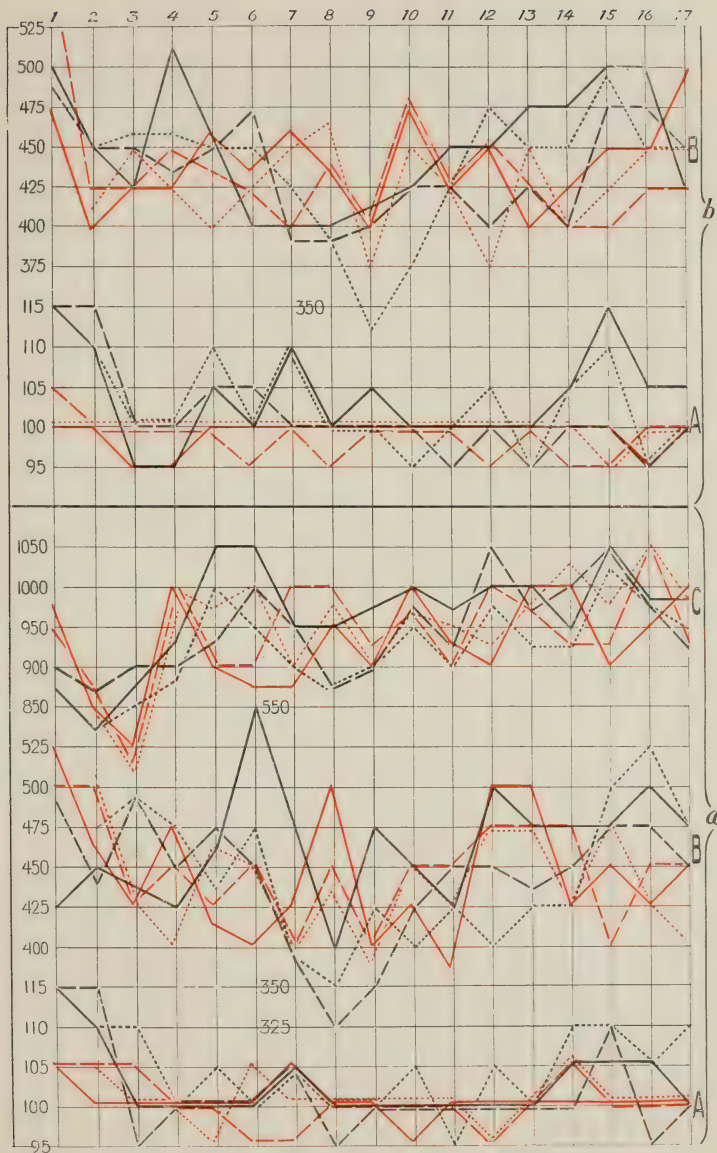
A straight black line separates the results obtained with (a) from those obtained with (b); an irregular line separating (b) from (c) begins between numbers 95 and 1050, on Plate 2.

The zigzag character of the curves shows the very variable results obtained, while the fact that the red and black lines correspond so rarely shows that the medium used and the temperature of the incubator were not influencing factors since these were identical for both. One might conclude that culture (b), the second obtained from Washington and grown alternately on agar and in bouillon, gives the least variable results. The difference between this and the others, however, is only slight and unimportant.

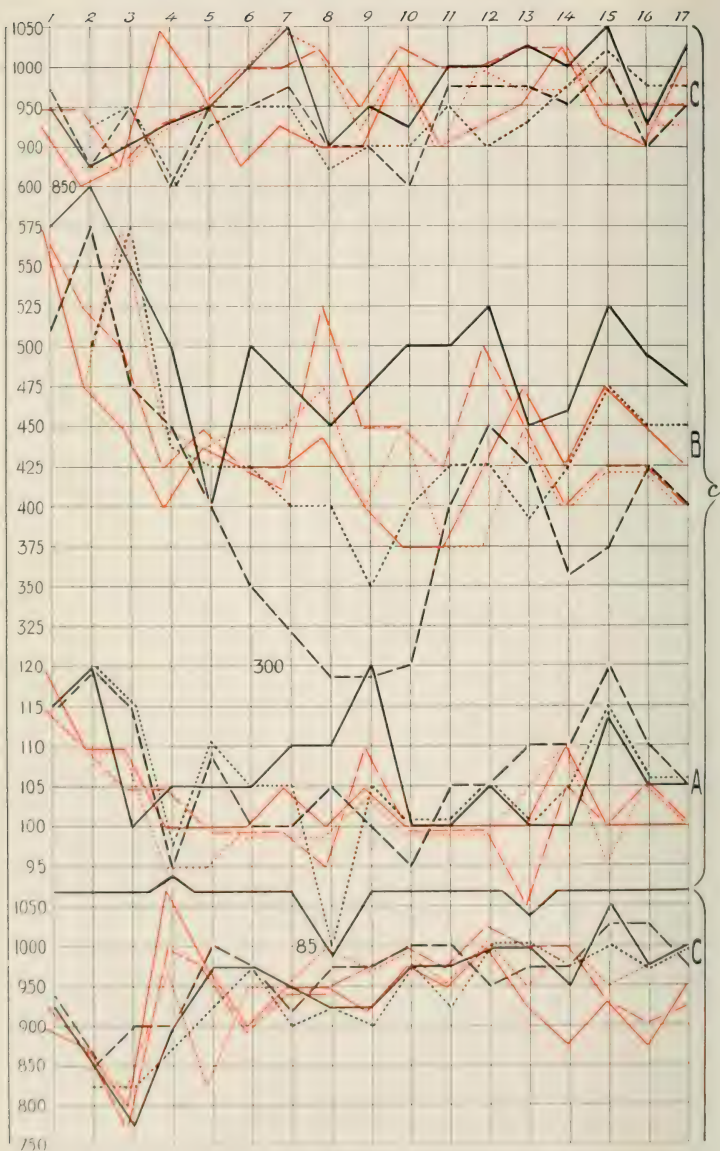
We are not prepared at this time to explain many of the results shown in the above tables and curves. We may conclude, however, that certain unknown conditions very profoundly affect the resistance of the test organism. In fact, it seems almost unquestionable that the different and varying resistance of the test organism is responsible for more of the variable results than is the technique of testing.

We have shown that the Hopkins culture is more resistant than culture No. 0190 in either medium. The temperature at which the test is carried out is of secondary importance, but some certain temperature should be adopted for the sake of uniformity. The amount of the culture medium used should not be less than 5 cc., but it is only in the case where an amount of the disinfectant carried over in a loopful might be antiseptic in 5 cc. that any larger quantity is necessary. The amount of a healthy broth culture of the organism which should be inoculated into the 5 cc. of disinfectant is unimportant within reasonable limits. Results obtained by using 0.1, 0.2, 0.4 cc. did not vary more than the variation due to the personal factor.

The point at which the comparison with standard is made seems unimportant and so little is gained by comparison at two points that one may be omitted to advantage. An average in the



Nº 1.



extremes of time rather than averaging the results at two times seems much more logical.

We wish to suggest, therefore, the following points which, as stated in the introduction, might be used to advantage in attempting to standardize disinfectants:

The Test Organism. There are some objections urged against the use of *B. typhosus*, but no other organism seems better adapted to the purpose. The Hopkins strain suggested by the Hygienic Laboratory is perhaps as satisfactory as any other, although one which is more sensitive to the disinfectants with a coefficient of 5 or over is more accurate since it shows finer shades of differences between samples. Culture No. 0190 has been in use many years, and while occasionally the bouillon culture has been noticed to change in its resistance, the agar culture seems to be exceptionally uniform.

Method of Growing. To obtain the greatest degree of uniformity in the vitality of the culture apparently requires very little attention other than that which is so essential in bacteriologic technique; namely, pure cultures, sterile apparatus, uniform temperature and medium in which to grow, and an occasional comparison of the bouillon culture with a fresh culture from the agar.

The Culture Medium. A medium containing more nutrient than that adopted by the Hygienic Laboratory seems to give more uniform results.

Proportion of Culture to Disinfectant. An average amount of culture for inoculating, such as 0.2 cc. per tube of disinfectant, is sufficient for obtaining good subcultures, is easily measured, and is not an excess.

Dilutions of the Disinfectant. The dilutions of the disinfectant to be tested are logically those which are approximately proportioned to the dilutions of the standard. If phenol is the standard and the dilutions increase by addition of 10, a disinfectant with a coefficient of 2 may have its dilutions increase by 20; if its coefficient is 5, by 50; if 10, by 100.

Loops for Transferring Subcultures. These should be of No. 23 U. S. gauge platinum wire, the loop being 4 mm. inside diameter. The means by which they are sterilized can be left

to the ingenuity of the individual worker. See *American Journal of Public Health*, Vol. 3, No. 6.

The Temperature During the Test. Any convenient room temperature such as 20° to 22° C. should be adopted and maintained by any convenient method during the test. That suggested in the article previously cited (*American Journal Public Health*, Vol. 3, No. 6) is very satisfactory.

Seeding Tubes. While one more surely guards against contaminating the subcultures by using the narrow seeding tubes recommended in both the Parke, Davis & Co. and the Rideal-Walker Methods, the wide tubes suggested by the Lancet Commission and adopted in the Hygienic Laboratory Method are more convenient, and in a laboratory where contaminating influences are at a minimum the use of wide seeding tubes is recommended.

Time of Contact Between Organism and Disinfectant. An average time of 5 minutes after which all organisms should be dead seems a logical time limit for the reaction to take place, and since it is convenient to use a difference of $2\frac{1}{2}$ minutes between times of subculturing the inoculated dilutions of the disinfectant, the logical way is to accept for comparison between sample and standard those dilutions of each which fail to kill the organism in 5 minutes but which contain no live organisms in the $7\frac{1}{2}$ -minute subculture. All other dilutions and times of subculturing are non-essential under these circumstances and to eliminate them shortens and simplifies the process very materially. When subcultures are taken at only the two times, namely, after 5 minutes and $7\frac{1}{2}$ minutes' contact with the disinfectant, one person can inoculate five dilutions in $2\frac{1}{2}$ minutes, allowing one-half minute for each inoculation. Then, after a wait of $2\frac{1}{2}$ minutes, one proceeds with the subculturing, planting one from each seeding tube in succession and immediately taking a second subculture from each. The first test of a disinfectant whose coefficient is not known can be made with so wide a range of dilutions that its character can be determined; then the second test can be made in comparison with the appropriate standard. Ten dilutions should be sufficient in the second test to cover the necessary range both for the sample and the standard. With an assistant to shake the seeding tube after it is inoculated and to

aid in subculturing, 15 seconds is sufficient time for each operation and ten tubes can be inoculated and two subcultures taken from each in 10 minutes.

The Standard. It has been noticed repeatedly that changes in the resistance of the test organism toward coal-tar disinfectants, having coefficients of 5 or over, are not accompanied by a corresponding change in its resistance toward phenol. The critical dilution of the latter appears not to fluctuate nearly so much as that of the coal-tar disinfectants with different strains of the culture and at different times of testing. It seems advisable, therefore, to compare disinfectants with standards of similar origin and approximately the same coefficient.

TABLE VII.
ILLUSTRATION OF PROPOSED METHOD.

Hopkins' Culture,
Liebig's Extract Medium,
Temperature—20° C.
Amount of Culture—0.2 Cc.

Minutes	Phenol		Disinfectant A.*				Disinfectant B.*			
7½	—	+	—	—	+	+	—	—	+	+
	110	120	22	24	26	28	18	19	20	21
5	+	+	—	+	+	+	—	+	+	+

Coefficient A=22—

Coefficient B=17+

Steps in the process and time consumed for each.

Inoculating 2½ minutes.

Wait 2½ minutes.

First Subculture at 5 minutes.

Second Subculture at 7½ minutes.

*Dilutions of A and B are in hundreds.

If we have succeeded in proving the importance of the test organism and of the standard and the minor importance of many details in obtaining uniform results when testing disinfectants, and if our suggestions by which the process may be materially shortened and simplified receive consideration, the object of this paper will have been attained.

REPRINTS OF PUBLICATIONS FROM THE RESEARCH LABORATORY, PARKE, DAVIS & CO., DETROIT, MICH.

The present system of collecting reprints of articles published from the Research Laboratory was begun in 1912. Reprints of the following articles published subsequent to that time are available and will be sent upon request.

1. On the Administration of Diphtheria Toxin in a Collodion Sac. By E. C. L. Miller. (*Journal of Infectious Diseases*, Vol. 8, January, 1911, pp. 50-65.)
2. A Further Contribution to Our Knowledge of Insecticides—Fumigants. By Chas. T. McClintock, H. C. Hamilton and F. B. Lowe. (*Journal of the American Public Health Association*, Vol. 1, April, 1911, pp. 227-238.)
3. Duboisia Hopwoodii—A Histological Study. By Oliver A. Farwell. (Reprinted from *Merck's Report*, Vol. 20, May 1, 1911.)
4. Etiology of Canine Distemper. By Newell S. Ferry. (*Journal of Infectious Diseases*, Vol. 8, June, 1911, pp. 399-420.)
5. The Resistance of Smallpox Vaccine to the Coal-tar Disinfectants. By Chas. T. McClintock and Newell S. Ferry. (*Journal of the American Public Health Association*, Vol. 1, June, 1911, pp. 418-419.)
6. Production of Immunity with Over-Neutralized Diphtheria Toxin. By Chas. T. McClintock and Newell S. Ferry. (*Abdruck Aus Dem Centrblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Originale, Bd. 59, July 15, 1911, pp. 456-464.)
7. Soaps from Different Glycerides—Their Germicidal and Insecticidal Values Alone and Associated with Active Agents. By H. C. Hamilton. (*Journal of Industrial and Engineering Chemistry*, Vol. 3, August, 1911, pp. 582-584.)
8. The Sleepy Grass of New Mexico: A Histological Study. By Oliver A. Farwell. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-273.)
9. Some Observations on the Physiological Action of Sleepy Grass. By A. W. Lescohier. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-275.)
10. An Investigation of the Depressor Action of Pituitary Extracts. By Carey P. McCord. (*Archives of Internal Medicine*, Vol. 8, November, 1911, pp. 609-620.)
11. The Physiology of the Pituitary Gland and the Action of Its Extracts. By Carl J. Wiggers. (*American Journal of Medical Sciences*, Vol. 141, April, 1911, pp. 502-515.)
12. A Physiological Investigation of the Treatment of Hemoptysis. By Carl J. Wiggers. (*Archives of Internal Medicine*, Vol. 8, 1911, pp. 17-38.)
13. Notes on Catgut Sterilization: A Preliminary Report. By Willard H. Hutchings. (*Annals of Surgery*, Vol. 54, July, 1911, pp. 693-695.)
14. The Relations of Pyogenic Microorganisms to the Etiology and Treatment of Skin Diseases. By Henry Rockwell Varney. (*Ohio State Medical Journal*, December, 1911.)
15. A Micrococcus with Unusual Characteristics as a Factor in a Resistant Dermatoses Resembling Acne Vulgaris. By Henry Rockwell Varney and L. T. Clark. (*Journal of Cutaneous Diseases*, Vol. 30, February, 1912, pp. 72-78.)

16. Serum Treatment of Hemorrhage and Blood Dyscrasias. By A. W. Lescohier. (*New York Medical Journal*, Vol. 95, February 3, 1912, pp. 223-229.)
17. Further Studies on the Bacillus Bronchicanis, the Cause of Canine Distemper. By Newell S. Ferry. (*American Veterinary Review*, Vol. 41, April, 1912, pp. 77-79.)
18. The Pharmacopœial Requirements for Cannabis Sativa. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, March, 1912, pp. 200-203.)
19. The Heart Tonic Unit. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 84, March, 1912, pp. 97-103.)
20. Studies on the Etiology of Equine Influenza. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, April, 1912, pp. 185-197.)
21. A Method for the Bacteriological Standardization of Disinfectants. By Tatsuzo Ohno and H. C. Hamilton. (*American Journal of Public Health*, Vol. 2, May, 1912, pp. 331-338.)
22. Physiological Testing. By E. M. Houghton. (*American Druggist*, July and September, 1911, and January and April, 1912.)
23. Bacillus Bronchisepticus (Bronchicanis) : The Cause of Distemper in Dogs and a Similar Disease in Other Animals. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, July, 1912, pp. 376-391.)
24. On Feeding Young Pups the Anterior Lobe of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 30, July, 1912, pp. 352-357.)
25. A Practical Portable Incubator. By Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Original, Bd. 65, Heft 4/5, 1912, pp. 412-413.)
26. Tobacco Extracts: Their Comparative Values as Insecticides. By W. O. Hollister. (*Journal of Economic Entomology*, Vol. 5, June, 1912, pp. 263-267.)
27. The Pharmacological Assay of Pituitary Preparations. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, October, 1912, pp. 1117-1119.)
28. Pituitary Extracts in Obstetrics and Gynecology. By A. W. Lescohier and O. E. Closson. (*Journal of the Michigan State Medical Society*, Vol. 11, October, 1912, pp. 650-657.)
29. Biological Products—Veterinary. By Robert H. Wilson. (*American Veterinary Review*, Vol. 41, September, 1912, pp. 668-681.)
30. The Isolation and Cultural Characteristics of Bacillus Acne. By Edwin M. Stanton. (*Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Original, Bd. 66, Heft 5/7, 1912, pp. 386-389.)
31. Studies on Hog Cholera. By Walter E. King and Robert H. Wilson. (*Journal of Infectious Diseases*, Vol. 11, Nov., 1912, pp. 441-458.)
32. Studies on the Virus of Hog Cholera. By Walter E. King and F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 39-41.)
33. The Physiological Activity of Cannabis Sativa. By H. C. Hamilton, A. W. Lescohier and R. A. Perkins. (*Journal of the American Pharmaceutical Association*, Vol. 2, Jan., 1913, pp. 22-30.)
34. The Iodine Content of the Small, Medium and Large Thyroid Glands of Sheep, Beef and Hogs. By T. B. Aldrich. (Original Communications, Eighth International Congress of Applied Chemistry, Vol. XIX, 1912, pp. 9-14.)

35. Studies on the Virus of Hog Cholera. By Walter E. King and Robert H. Wilson. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 16, Heft 3, 1913, pp. 367-376.)

36. On the Cultivation of the *Treponema Pallidum* (*Spirochæta Pallida*). By F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 55-67.)

37. Studies on the *Gonococcus*, I. By Carl C. Warden. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 93-105.)

38. Studies on the Virus of Hog Cholera. By Walter E. King, F. W. Baeslack and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 12, March, 1913, pp. 202-205.)

39. *Bacillus Bronchisepticus*—Its Relation to Canine Distemper. By N. S. Ferry. (*American Veterinary Review*, Vol. 43, April, 1913, pp. 16-30.)

40. Drug Influence on Extrasystoles of the Mammalian Heart. By Carey P. McCord. (*Interstate Medical Journal*, Vol. 19, Oct., 1912, pp. 870-880.)

41. The Employment of Protective Enzymes of the Blood as a Means of Extracorporeal Diagnosis. I.—Sero-Diagnosis of Pregnancy. By Carey P. McCord. (*Surgery, Gynecology and Obstetrics*, Vol. 16, April, 1913, pp. 418-421.)

42. Tribromo-tert-Butyl Alcohol, $C_4H_7OBr_3$. By T. B. Aldrich. (*Journal of the American Chemical Society*, Vol. 33, March, 1911, pp. 386-388.)

43. On Feeding Young White Rats the Posterior and the Anterior Parts of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 31, Nov., 1912, pp. 94-101.)

44. The Rationale of the Use of Adrenalin in the Treatment of Asthma. By Carey P. McCord. (*Medical Record*, Vol. 83, March 8, 1913, pp. 431-432.)

45. Standardization of Disinfectants: Some Suggested Modifications. By H. C. Hamilton and T. Ohno. (*American Journal of Public Health*, Vol. 3, June, 1913, pp. 582-588.)

46. Preventive Measures Against Equine Influenza Based on Its Bacteriology. By N. S. Ferry. (Report of the Proceedings of the United States Live Stock Association, December, 1912, p. 127.)

47. Correcting Water. By H. C. Hamilton. (*Bulletin of Pharmacy*, Vol. 27, August, 1913, pp. 330-335.)

48. Duration of Immunity Following Small-pox Vaccination. By A. W. Lescohier. (*Journal of the American Medical Association*, Vol. 66, Aug. 16, 1913, page 487-490.)

49. On Crystalline Kombe-Strophanthin. By D. H. Brauns and O. E. Closson. (*Journal of the American Pharmaceutical Association*, May, June and July, 1913, Vol. 2.)

50. A Comparative Study of Antigens for the Wassermann Reaction. By H. R. Varney and F. W. Baeslack. (*Journal of the American Medical Association*, Vol. 66, Sept. 6, 1913, pp. 754-757.)

51. The Treatment of Tetanus. By Charles T. McClintock and Willard H. Hutchings. (*Journal of Infectious Diseases*, Vol. 13, Sept., 1913, pp. 309-320.)

52. *Spirochæta Suis*, Its Significance as a Pathogenic Organism. Studies on Hog Cholera. By Walter E. King and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 13, Nov., 1913, pp. 463-498.)

53. Time Recorder for Kymograph Tracings. By Oliver E. Closson. (*Journal of Pharmacology and Experimental Medicine*, Vol. 5, Jan., 1914, pp. 235-238.)

54. U. S. P. Menstrua. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 86, Feb., 1914, pp. 56-61.)

55. Numerical Variations of the White Blood Cells in Mice Inoculated with Transplantable Adenocarcinoma. By F. W. Baeslack. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 20, Heft 5, 1914, pp. 421-435.)

56. A Study of the Germicidal Action of the Ultraviolet Rays. By E. M. Houghton and L. Davis. (*American Journal of Public Health*, Vol. 4, March, 1914, pp. 224-240.)

57. Some Phenomena Involved in the Life History of *Spirochaeta Suis*—Studies on Hog Cholera. By W. E. King and R. H. Drake. (*The Journal of Infectious Diseases*, Vol. 14, March, 1914, pp. 246-250.)

58. The Sterilization of Adrenalin Solutions. By L. W. Rowe. (*American Journal of Pharmacy*, Vol. 86, April, 1914, pp. 145-149.)

59. Infection and Immunity: A Review. By N. S. Ferry, Ph.B., M.D. (*Journal of the American Pharmaceutical Association*, Vol. 3, April and May, 1914.)

60. Disinfection—What Disinfectant is the Most Generally Applicable for Clinical, Surgical and Sanitary Purposes? By H. C. Hamilton. (*Therapeutic Gazette*, Vol. 38, May, 1914, pp. 311-315.)

61. Study of the Bacteriology of the Posterior Nasopharynx in Scarlatina. By N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, pp. 934-935.)

62. Some Experiences with Bacterial Vaccines in Scarlatina. By Guy L. Kiefer, M.D., D.P.H., and N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, p. 936.)

63. A Sero-enzyme Test for Syphilis. By F. W. Baeslack, M.A., M.D. (*The Urologic and Cutaneous Review*, Vol. 18, May, 1914, pp. 234-238.)

64. Bacteriology and Control of Acute Infections in Laboratory Animals. By N. S. Ferry, Ph.B., M.D. (*Journal of Pathology and Bacteriology*, Vol. 18, 1914.)

65. The Bacteriological Standardization of Disinfectants. By H. C. Hamilton and Tatsuzo Ohno. (*American Journal of Public Health*, Vol. 4, No. 6.)

THE PINEAL GLAND IN RELATION TO SOMATIC, SEXUAL AND MENTAL DEVELOPMENT.*

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From tumors involving the pineal gland, two distinct systems of symptoms and signs ensue, the neurologic and the metabolic. The neurologic manifestations arise from the encroachment of the neoplasm on the intracranial contents and are indications of changes in pressure, in placement and of destruction of tissue. Such changes are the consequence of pineal tumors at any age of the patient; but in pineal tumors appearing in prepuberal life a second group of changes arise, the metabolic. These metabolic alterations are referable to disturbance in the gland's secretory function. Apparently only in young males is this syndrome complete.¹ It consists of (1) early sexual development evidenced in the enlarged sex organs, pubic hair, general body hair, early changes in voice; (2) precocious mental development evidenced in the maturity of thought and speech; and (3) general body overgrowth to the extent that a child of 5 or 6 years may have the appearance of a child of 11 or 12.

A case reported by Machell² presents these changes in a striking manner. The patient was a boy less than 6 years old at the time of the publication. At the age of 5 months there was pubic hair, erections at 17 months, emissions at 30 months. The patient's weight was 7½ pounds above normal at 4 months of age, 12 pounds in excess at 8 months, 20 pounds in excess at 3 years. When the patient was 44 months old, his height was 8½ inches above normal for a child of that age. At 48 months the circumference of the head was over 2 inches in excess of normal. The voice was a deep bass. Mental precocity was very marked and the general bearing and language was that of a much older person.

On account of the difficulties attending experimentation on this vestigial organ, the clinical findings with subsequent necropsy

*Read before the Section on Pathology and Physiology at the Sixty-Fifth Annual Session of the American Medical Association, Atlantic City, N. J., June, 1914.

1. For review of clinical cases see Bailey, Pearce, and Jelliffe, Smith Ely: Tumors of the Pineal Body, *Arch. Int. Med.*, December, 1911, p. 851. For Physiology and Anatomy, see Vincent Swale; *Internal Secretion and Ductless Glands*, 1912.

2. Abstract taken from *Medical Chronicle*, 1912, lvii, 154.

records have been the prime factor in the formulation of the prevailing ideas as to this gland's functions. The conception of this gland's function, however, has in part been developed from laboratory studies, notably from the results concomitant to the extirpation of the organ. This has been attempted frequently, but the situation of the gland is such that in the greater number of instances death followed the operative procedure, from hemorrhage or injury to the vermis or the occipital lobes. By operating on a large number of animals some workers have had a few animals survive. No changes attended the removal of the gland by Biedl,³ Dandy,⁴ and Exner and Boese,⁵ but Foa⁶ ablating the gland in chicks and Sarteschi⁷ in young rabbits and puppies report the production of the precocious macrogenitosomatic syndrome.

The publications growing out of these several clinical and laboratory studies have given rise to conceptions of this organ and its functions that may be thus epitomized:

The pineal body (epiphysis cerebri), probably the remains of a parietal eye, is situated just beneath the splenium of the corpus callosum, resting on the anterior quadrigeminate bodies, and is attached by its base to the habenular commissure. The gland varies in size, shape and pigmentation and does not stand in any proportional relation to the size of the brain or size of the body. The pineal body contains glandular elements, but these are few and not well defined. The greatest postnatal development is in the first years of life, and as far as is known the gland is only functionally active in the prepuberal life. On the assumption that tumors destroy the gland and deprive the body of the substances generated by it, the function of the gland is generally described as retarding and holding in abeyance too rapid development in childhood, of the body, mind and sexual characteristics. When precocity in development appears in conjunction with pineal tumors, it is attributed to lack of glandular secretion.

Below are recorded some findings from experimental work that are entirely in accord with the results obtained by Dana and Berkeley.⁸ Contrary to the view that precocious development is due to pineal deficiency (hypopinealism) the same precocity in the development has been brought about by the oral administration of pineal gland tissue.

3. Biedl: *Innere Sekretion*, 1910.

4. Dandy, cited by Cushing: *Pituitary Gland and Its Disorders*, p. 283.

5. Exner and Boese: *Deutsch. Ztschr. f. Chir.*, 1910, cvii, 182.

6. Foa: *Arch. Ital. de Biol.*, 1912, lvii, 233.

7. Sarteschi: *Pathologia*, 1913, p. 707.

8. Dana and Berkeley: *Med. Rec., N. Y.*, 1913, lxxxiii, 835.

EXPERIMENTAL DATA.

The general plan of work entailed in this study has been to feed to very young animals minute quantities of pineal tissue and to record the weight changes, sexual differences, and in the case of dogs increased mentality, over control animals maintained under otherwise identical conditions. One hundred and ten guinea-pigs, eighteen puppies, fourteen adult dogs and sixteen chicks have been under observation.

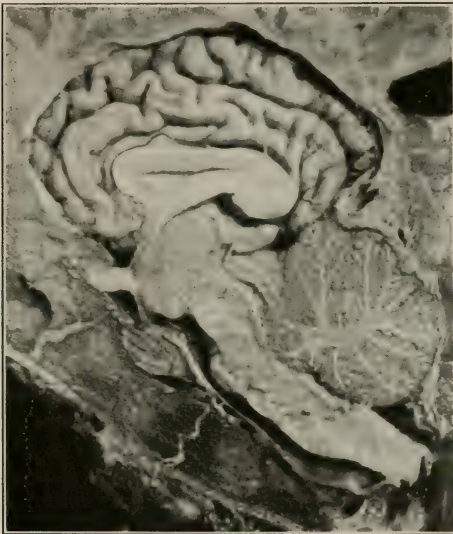


Fig. 1.—Position, relations and relative size of the pineal gland. Taken from beef 3 years of age.

Fresh pineal glands from cattle have been employed. These were in part from veal, in part from young adults approximating 3 years, and in part from the general run of cattle from the abattoirs. The glands averaged in weight (on weighing sixty glands) 2.14 grains. There were in the many thousand glands making up the several pounds that have been used, marked variations in size, shape and melanopigment. This pigment, which was present

in a high percentage of glands, involves chiefly the encapsulating tissue.

For feeding experiments the glands were prepared for permanent use in the following manner: The fresh glands were rinsed free of blood and stripped of adherent tissue. They were ground to a fine paste in the Latapie grinder, and without drying the paste was admixed with milk sugar in such quantity that 1/2 grain milk sugar represented 10 mg. pineal tissue. The mass was made into 1/2 grain tablets and quickly dried at room temperature.

Early in the work it was apparent that the more striking results were being obtained from the animals fed with pineal substance from cattle not having reached adult life. Efforts were then made to establish quantitatively the activity and the identity of the various experimental lots of pineal preparations by employing the methods commonly used in testing the activity of endocrinous derivatives. Although certain cardiovascular changes regularly follow the intravenous administration of pineal extracts to dogs, the extent of these changes is not a measure of the activity of the gland as a stimulator of growth.

On the hypothesis that precocious development is due to hypopituitarism, the first work was done in anticipation that feeding would retard development and prolong the presexual life. This was begun on two chicks incubated in the laboratory. Beginning at the age of two days one was fed 10 mg. veal pineal tissue, three times weekly, the other (and in all cases of controls) was fed a blank tablet of milk-sugar. The difference in growth should be noted (Table I).

TABLE I.—RESULTS OF FEEDING PINEAL GLAND TO CHICKS.

	Third Week. gm.	Eighth Week. gm.	Ninth Week. gm.	Twelfth Week. gm.
Pineal	219.5	557.7	805	925
Control	92.5	286.5	560	700
Difference	127.0	271.2	245	225

The striking disproportion in size and the marked skeletal overgrowth, making the large chick very awkward in his movements, soon made these chicks a laboratory curiosity, but the smaller number and the different sex did not justify any inference as to the influence of the pineal feeding. The results, however,

were so striking that at once work was instituted in a more extensive way.

A lot of fifty guinea-pigs in the second week of life was selected and divided into test and control groups. The test pigs were fed daily 10 mg. veal pineal tissue. The controls were fed a $\frac{1}{2}$ -grain milk-sugar tablet. Other conditions for the two lots were identical. The results obtained are given in Table 2.

TABLE 2.—RESULTS OF FEEDING PINEAL GLAND TO YOUNG GUINEA-PIGS.

Control. 25 Pigs. Average Initial Weight, 201.2 gm. Age, 2 Weeks.			Pineal. 25 Pigs. Average Initial Weight, 204.3 gm. Age, 2 Weeks.		
Weeks.	Aver. Wt. gm.	Gain. gm.	Weeks.	Aver. Wt. gm.	Gain. gm.
1	198.7	-2.5	1	213.2	8.9
2	220	21.3	2	236.8	23.6
3	226.8	6.8	3	247.6	10.8
4	251	24.2	4	273.2	25.6
5	265.3	14.3	5	299.9	26.7
6	265.7	0.4	6	311.1	11.2
7	292.2	26.5	7	338	26.9
8	306	13.8	8	361.4	23.4
9	316.3	10.3	9	365.5	4.1
10	356.5	40.3	10	410.8	45.3
Average weight at end of tenth week.....			Average weight at end of tenth week.....		
356.6 gm.			410.8 gm.		
Average initial weight.....			Average initial weight.....		
201.2 gm.			204.3 gm.		
Average gain.....			Average gain.....		
155.4 gm.			206.5		
Gain, per cent.....			Gain, per cent.....		
77.0			100.0		

Excess gain of pineals over controls, 23 per cent.

This excess in weight of pineal-fed guinea-pigs over their controls is a symmetrical overgrowth. There is some increased adipose tissue, but this is generally distributed and not localized in any one region of the body. At no time has it been possible to continue this excessive growth above normal adult size. As the animals approach adult size the pineal feeding is less effective and after full maturity is attained is without effect. There has been no tendency to gigantism.

As a step toward determining the metabolic differences, quantitative urinalyses were made on twenty-four-hour composite samples of urine from the two groups.⁹ With proper regard for the numerous other factors that might vitiate the results obtained from uninalyses, there is a suggestive difference in the two urines as may be seen in the summary given in Table 3.

⁹ I am under obligations to Mr. Lewis Davis for these analyses.

TABLE 3.—SUMMARY OF ANALYSIS OF URINES.

	Pineal	Control.
Total volume collected (24 hours).....	187 c.c.	160 c.c.
Specific gravity (15.5 C.).....	1.016	1.019
	Per Cent.	Per Cent.
Total solids.....	2.51	3.02
Water.....	97.49	96.98
Total mineral matter.....	1.21	1.37
Total nitrogen.....	0.628	0.872
Total creatinin.....	0.018	0.025
Phosphoric acid (as H_3PO_4).....	0.065	0.043
Calcium.....	0.016	0.018
Magnesium.....	0.020	0.018
Alkalinity (as Na_2CO_3).....	0.053	0.037

Chicks.—A lot of fourteen chicks was secured at the age of one week. At so early an age sex could not be determined, and the lot was divided into test and control groups without knowledge as to grouping by sex. The test chicks were placed on veal pineal tissue for one week with a resultant greater growth than controls. For the next four weeks they were fed pineal tissue from old cattle, without gaining. On being placed on the original veal preparation the test chicks again grew in excess over controls.

Dogs.—The eighteen dogs employed represent four litters. Infection of these puppies with distemper interfered seriously with the weight charts of these animals. So long as infections could be kept out of the animal quarters, the test animals outgrew the controls, but the wasting from infections interfered seriously with average results over prolonged periods. It was in connection with puppies that some differences in intelligence were observed. No great import is attached to these observations, but it was noted that the pineal-fed dogs were about one month ahead of the others in their habits. They were the first to learn to lap milk, the first to respond to a call, the first to be able to find their way back to the kennel. When work on these animals was discontinued, only the pineal-fed animals were in demand as pets, and those choosing them did so without any knowledge as to differences in feeding. Of much more importance as regards increased intelligence are the favorable results reported by Berkeley,¹⁸ who administered pineal tissue to mentally defective children and performed Binet tests as a criterion of mental advancement.

Precocious Sexual Development.—A group of forty-eight

¹⁸ Berkeley: Mail, Rev., New York, 1914. DAVIS, D.

guinea-pigs was divided into test and control lots. There was an equal number of males and females in each lot, but the males and females were separated. The test pigs were fed veal pineal tissue in 10 mg. amounts daily. Feeding was begun when the animals were 2 weeks old and continued for nine weeks. The males and females of each group were then placed together in breeding-pens. As a measure of any difference in sexual development it was thought desirable to note the date of birth of young in the two lots. As the end of the approximate gestation period approached, these animals were observed as to the date of giving birth to young. All except two of the pineal-fed pigs gave birth



Fig. 2.—Effect on growth of feeding pineal-gland tissue to chicks. The larger chick was fed 10 mg. pineal tissue three times weekly.

to young before the first of the controls.† Fourteen days elapsed between the birth of the young of the first pineal-fed pig and the first control pig. In all cases the young were normal and in no wise different from any other young pigs.

COMMENT AND SUMMARY.

In the foregoing records of experiments, it may be observed that some of the changes generally attributed to deficiency of pineal secretion may be produced by supplying an added amount of pineal substance. In an effort to reduce to rationality these

†In a second series of fifty guinea-pigs observed as to sexual differences, the males and females have been kept together from birth. Some of the females to whom pineal gland tissue has been fed have already given birth to young and many others are within the last ten days of gestation. With the exception of one pig, the control animals evince no signs of pregnancy.

identical findings derived from two opposing sources, the so-called destructive neoplasms of the pineal gland on the one hand, and the feeding of the gland on the other, there arise two possibilities: First, this syndrome may appear from disrupting the general endocrinous balance from either increasing or decreasing the amount of pineal secretion available for the body's use. These secondary changes in the other endocrinous glands are now in the course of investigation. Second, the cells of the neoplasms involving the pineal gland may retain some of the metabolic and other functional characteristics of the normal pineal cell from which they were derived, and the peculiar body, sexual and mental changes in patients with such tumors are all manifestations of increased rather than decreased pineal activity. One of the most frequently occurring lesions of the pineal is the adenoma, and there is abundant evidence that at times cells of



Fig. 3.—Effect on growth of guinea-pigs produced by feeding pineal-gland tissue. Control pigs to left, test pigs to right.

adenoma functionate after the manner of the cells from which they arise. In adenoma of the liver in cases reported by Weber,¹¹ Rolleston,¹² Wheeler¹³ and Ribbert,¹⁴ distinct bile secretion by tumor-cells has been pointed out. Ribbert established that the bile present in such tumors was not the bile of icterus from necrotic liver-tissue by demonstrating that the scirrhous encapsulating tissue was free from bile-stain and that the bile was confined to the liver-like cells of the active tumor. In at least one case, a metastasis in the lung from the liver secreted bile. In this connection it is significant that the functioning glandular cells of typical thyroid structure have been found in thyroid metastases in bone tissue. Furthermore, in myeloma of the bone-marrow

11. Weber: *Proc. Roy. Soc. Med. (Path. Sec.)*, 1910, iii, 147.

12. Rolleston: *Diseases of the Liver*, 1905.

13. Wheeler: *Guy's Hosp. Rep.*, 1909, lxiii, 225.

14. Ribbert: *Deutsch. med. Wochenschr.*, 1909, xxxv, 1607.

the cytoplasm¹⁵ of the tumor-cells contain the granules that characterize normal myelocytes, that is, the tumor to a certain extent assumes the function of the bone-marrow. All considered, it is perceived that functional activity of tumor-cells is *not* infrequent. Germane to the present contention is the statement by Hinds Howell¹⁶ in describing the characteristics of the cells of the pineal tumors of his three cases. He says:

A noteworthy feature is the similarity of these tumor-cells in many instances to those of the normal pineal gland.

The results of this work lead me to the conclusion that the administration of minute quantities of pineal tissue from young animals to young animals stimulates rapid growth of the body, but not beyond normal size. Also there are less well-established indications of precocity of mental and sexual development.

15. Weber and Ledingham: Proc. Roy. Soc. Med. (Path. Sec.), 1909, ii, 206.

16. Howell, Hinds: Proc. Roy. Soc. Med. (Neurolog. Sec.), 1910, iii, 65.

THE SERO-ENZYME TEST FOR SYPHILIS*

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Through the parenteral incorporation of foreign or changed protein into the organism we may observe the formation of enzymes in the blood of the body. These enzymes do not occur in the blood normally, but their presence is contingent on the entrance of blood-foreign substances. Conversely we are able to prove the occurrence of these enzymes in the blood by dialyzing such serum against the protein which gave rise to the enzymes.

The formation of these enzymes may be stimulated in the experiment at the will of the investigator, or it may take place in diseases of infectious origin, when the organisms themselves or the products of their action on the cells of the body, or both, are swept into the blood-stream and there cause the development of ferments which are capable of breaking up the complex foreign substances into their simpler non-specific component parts, which are either assimilated or thrown off. In this respect the normal and abnormal functions of the body differ quantitatively—for while, under normal conditions, the cells of the body split the complex specific molecules into simpler non-specific parts, there is added, under certain conditions, either physiologic or pathologic, the action of the ferments present or increased in the blood-serum which have the same function, namely, to split specific bodies into their non-specific components.

The work of Abderhalden has called attention to the formation and action of these ferments in pregnancy, in the course of which either chorionic epithelium or the products of the active metabolism as occurring in the syncytium may find their way into the blood-stream. Later Abderhalden extended his investigations to the diagnosis of neoplasms.

The controversy concerning the applicability of the dialyzing

* Read before the Section on Dermatology at the Sixty-Fifth Annual Session of the American Medical Association, Atlantic City, N. J., June, 1914.

method for diagnostic purposes has found a fertile field in the sero-enzyme reaction for cancer, and a large number of observations have been made, which speak against the reliability of this method of diagnosis in this disease. As far as it has been possible to determine, the failures may be due to a variety of causes, aside from possible errors in technic.

It is practically impossible to obtain cancer-tissue free from the connective-tissue stroma. The reaction for connective tissue is the same. This may be the reason why certain serums give positive reactions in other conditions than those clinically apparent. As the cancer invades an organ, there is usually degeneration of the neighboring tissue-cells and a consequent occurrence of protective enzymes in the serum of the patient against this cell protein. In addition, the cancer-cell may retain traces of the functional activity of the tissue from which it originated. Finally, the occurrence of protective enzymes in the serum is dependent on the breaking up of tissue in the blood and on the resorption of such broken-down tissue. Thus, an actively growing neoplasm may not give off cancer protein at all or may give it in such small quantities that the enzymes directed against it are not demonstrable. For these reasons the choice of the sero-enzyme diagnosis for neoplasms may not have been a happy one to bring the conflicting conclusions into any relation. The use of homologous cancer-tissue, that is, tissue from patients who had the new growth in the same relation as that of the suspected patient, may possibly shed some light on this line of work. At least these contradictory results are no reason for excluding this method of diagnosis from all clinical application.

The extraordinary significance of the work of Abderhalden from a clinical and purely scientific point of view should be a stimulus to further research to determine the factors which have given rise to these divergent results.

The infectious diseases offer a more promising field for this line of investigation, since, in the course of such diseases, the causative organisms find their way into the circulation and there give rise to the protective enzymes capable of digesting them. Aside from this, we have in syphilis a disease simulating many other conditions in which cell proliferation and destruction occur,

so that the resorption of these broken-down foci by the blood will further stimulate the formation of enzymes against the protoplasm of the cells undergoing degeneration as the result of the presence of the *Spirochæta pallida* in the body.

Two hundred serums, obtained mostly from patients of the outdoor clinics of St. Mary's Hospital, form the basis for this investigation. While, in a previous paper,¹ the majority of serums tested were obtained from known syphilitics, it has been my aim in this work to test serums of patients suffering from various complaints to determine the extent to which this reaction could be relied on as a diagnostic means.

The technic followed in the course of this work has been the same as was employed for the fifty-five cases already reported. The tissue employed was the same, namely, the "gummas" resulting from the inoculations of syphilitic tissue or blood into the testes of rabbits. These lesions become, in the course of a few transplantations from rabbit to rabbit, free from secondary infection. They are practically non-vascular and contain the *Spirochæta pallida* in large numbers.

Tissues from syphilitic patients, such as condylomas, gummas, etc., are usually secondarily infected and very vascular, for which reasons they are not suitable as tissues for the sero-enzyme reaction. The testicular tissue which has undergone mucoid degeneration due to the presence of the *Spirochæta pallida* is of special advantage in the sero-enzyme test in that we are making use of a highly specialized tissue, against which protective enzymes are not likely to be found in the serum of patients. Thus the sero-enzyme test becomes a specific biologic test for the diagnosis of this disease. While the Wassermann reaction is a valuable aid in the diagnosis of syphilis, the principles underlying this reaction are not yet understood after years of investigation and general use.

Table 1 gives the clinical diagnosis as well as the serologic findings of the two hundred cases.

Eighty-five, or 42.5 per cent, of the entire number of cases gave a history of specific infection. Table 2 gives the number of each case as well as the serologic findings.

1. Baeslack, F. W.: A Sero-Enzyme Diagnosis of Syphilis, THE JOURNAL A. M. A., March 28, 1914, p. 1002; Urol. and Cutan. Rev., May, 1914, p. 234.

TABLE 1.—DIAGNOSIS AND SEROLOGIC FINDINGS IN 200 CASES

Name	Case	Date	Age	Sex	Stage of Syphilis	History	Treatment	Wassermann Reaction	Sero-enzyme Test			Other Diseases and Complaints
									Syph.	Tuberc.	Colter	
A. K.	1	4-11	35	M.	Primary, 5 weeks	+	+	++	+			
D. D.	2	11-11	35	M.	Latent	+	+	++	+			
L. S.	3	11-11	35	M.	Primary, 4 weeks	+	+	++	+			
S. S.	4	4-4	17	F.	Cerebral	+	+	++	+			
Th. R.	5	11-11	17	M.	Gumma of testis	+	+	++	+			
A. Y.	6	9-14	50	M.	Latent	+	+	++	+			
A. C.	7	10-14	35	M.	Secondary	+	+	++	+			
Th. M.	8	16-14	35	M.	Latent	+	+	++	+			Headache
M. M.	9	16-14	43	M.	Latent	+	+	++	+			Uncontrolled shaking of hands
L. M.	10	17-14	33	M.	Primary, 5 weeks	+	+	++	+			
C. C.	11	17-14	38	M.	Primary, 6 weeks	+	+	++	+			
F. W.	12	17-14	34	M.	Gumma on arms	+	+	++	+			
M. D.	13	20-14	40	M.	Latent	+	+	++	+			
M. T.	14	20-14	30	M.	Latent	+	+	++	+			
A. B.	15	23-14	22	M.	Latent	+	+	++	+			
A. H.	16	23-14	23	M.	Ulcers in mouth	+	+	++	+			
N. H.	17	23-14	23	M.	Primary, 5 weeks	+	+	++	+			
N. B.	18	25-14	23	M.	Primary, 5 weeks	+	+	++	+			
L. R.	19	25-14	31	M.	Primary, 4 weeks	+	+	++	+			
A. R.	20	25-14	40	M.	Latent	+	+	++	+			
E. J.	21	27-14	35	M.	Cerebral lues	+	+	++	+			
H. D.	22	27-14	40	M.	Prim-second	+	+	++	+			Lupus vulgaris?
H. J.	23	27-14	31	M.	Latent	+	+	++	+			
F. J.	24	27-14	31	M.	Latent	+	+	++	+			Sore throat
E. G.	25	27-14	33	M.	Primary, 2 weeks	+	+	++	+			Pleurisy l. side
E. G.	26	27-14	33	M.	Has chills syphilitic	+	+	++	+			
L. Th.	27	27-14	33	M.	Latent	+	+	++	+			Headache
A. S.	28	27-14	39	M.	Latent	+	+	++	+			Chancroid?
H. R.	29	27-14	31	M.	Latent	+	+	++	+			
H. T.	30	27-14	32	M.	Latent	+	+	++	+			
K. D.	31	27-14	39	M.	Latent	+	+	++	+			
P. L.	32	27-14	45	M.	Primary	+	+	++	+			
Th. M.	33	27-14	60	M.	Cerebral lues	+	+	++	+			
S. M.	34	27-14	19	M.	Latent	+	+	++	+			
S. M.	35	10-14	35	M.	Latent	+	+	++	+			
S. M.	36	10-14	35	M.	Latent	+	+	++	+			
D. N.	37	10-14	38	M.	Latent	+	+	++	+			
N. M.	38	10-14	40	M.	Latent	+	+	++	+			
M. R.	39	10-14	42	M.	Ulcers on legs	+	+	++	+			
M. B.	40	10-14	36	M.	Tertiary	+	+	++	+			
F. C.	41	10-14	32	M.	Latent	+	+	++	+			
C. H.	42	10-14	32	M.	Latent	+	+	++	+			
Th. H.	43	1-11	50	M.	Cerebral syphilis	+	+	++	+			
A. N.	44	16-11	32	M.	Latent	+	+	++	+			
E. H.	45	16-11	31	M.	Latent	+	+	++	+			
A. H.	46	16-11	48	M.	Secondary	+	+	++	+			
A. J.	47	1-11	39	M.	Tuberc.	+	+	++	+			Pain in arms and legs

No.	Sex	Age	Initial symptoms	Duration of disease	Course	Result	Remarks
1	M.	48	3/18/14	56	Tuberc.	Primary	Scenic dementia
2	M.	49	3/19/14	56	Syph. neuritis	Primary	Mixed infection
3	F.	50	3/19/14	55	Syph. neuritis	Primary	Tuberculosis
4	F.	51	3/20/14	19	Syph. neuritis	Primary	Visceral phos
5	F.	52	3/21/14	43	Syph. neuritis	Primary	Sore throat
6	F.	53	3/21/14	39	Syph. neuritis	Primary	Stomach disturbances and falling of hair
7	F.	54	3/22/14	32	Syph. neuritis	Primary	Ulcers of legs
8	F.	55	3/23/14	26	Syph. neuritis	Primary	Periostitis on r. olecranon
9	F.	56	3/23/14	29	Syph. neuritis	Primary	Arthritis
10	F.	57	3/23/14	29	Syph. neuritis	Primary	Exostosis on r. tibia. Head-aches
11	F.	58	3/24/14	..	Syph. neuritis	Primary	Black-water fever
12	F.	59	3/25/14	27	Syph. neuritis	Primary	Pain in r. arm and leg
13	F.	60	3/25/14	19	Syph. neuritis	Primary	Frozen foot, refused to heal
14	F.	61	3/25/14	29	Syph. neuritis	Primary	Coryza
15	F.	62	3/25/14	31	Syph. neuritis	Primary	Pain in left shoulder
16	F.	63	3/25/14	25	Syph. neuritis	Primary	Epileptic
17	F.	64	3/25/14	43	Syph. neuritis	Primary	Gonorrhea
18	F.	65	3/25/14	38	Syph. neuritis	Primary	Ulcerations on l. arm and fingers
19	F.	66	3/25/14	29	Syph. neuritis	Primary	Erosion of post-nasal bones.
20	F.	67	3/25/14	19	Syph. neuritis	Primary	Ozena
21	F.	68	3/25/14	52	Syph. neuritis	Primary	Extoses of head
22	F.	69	3/27/14	..	Syph. neuritis	Primary	Tuberculosis
23	F.	70	3/27/14	29	Syph. neuritis	Primary	Cretion Alopecia
24	F.	71	3/30/14	26	Syph. neuritis	Primary	Gonorrhea
25	F.	72	3/30/14	42	Syph. neuritis	Primary	Pulm. H. advanced
26	F.	73	3/30/14	28	Syph. neuritis	Primary	..
27	F.	74	3/31/14	35	Syph. neuritis	Primary	..
28	F.	75	4/1/14	33	Syph. neuritis	Primary	..
29	F.	76	4/1/14	32	Syph. neuritis	Primary	..
30	F.	77	4/1/14	38	Syph. neuritis	Primary	..
31	F.	78	4/3/14	36	Syph. neuritis	Primary	..
32	F.	79	4/3/14	50	Syph. neuritis	Primary	..
33	F.	80	4/3/14	50	Syph. neuritis	Primary	..
34	F.	81	4/6/14	181	Syph. neuritis	Primary	..
35	F.	82	4/6/14	33	Syph. neuritis	Primary	..
36	F.	83	4/7/14	24	Syph. neuritis	Primary	..
37	F.	84	4/7/14	28	Syph. neuritis	Primary	..
38	F.	85	4/8/14	32	Syph. neuritis	Primary	..
39	F.	86	4/8/14	53	Syph. neuritis	Primary	..
40	F.	87	4/8/14	21	Syph. neuritis	Primary	..
41	F.	88	4/8/14	35	Syph. neuritis	Primary	..
42	F.	89	4/9/14	25	Syph. neuritis	Primary	..
43	F.	90	4/10/14	23	Syph. neuritis	Primary	..
44	F.	91	4/13/14	24	Syph. neuritis	Primary	..
45	F.	92	4/13/14	35	Syph. neuritis	Primary	..
46	F.	93	4/13/14	42	Syph. neuritis	Primary	..
47	F.	94	4/13/14	42	Syph. neuritis	Primary	..
48	F.	95	4/15/14	12	Syph. neuritis	Primary	..
49	F.	96	4/16/14	48	Syph. neuritis	Primary	..
50	F.	97	4/17/14	24	Syph. neuritis	Primary	..
51	F.	98	4/17/14	52	Syph. neuritis	Primary	..
52	F.	99	4/17/14	22	Syph. neuritis	Primary	..

TABLE 1.—DIAGNOSIS AND SEROLOGIC FINDINGS IN 200 CASES—(Continued)

Name	Case	Date	Age	Sex	Stage of Syphilis	History	Treatment	Wassermann Reaction	Sero-enzyme Test			Other Diseases and Complaints
									Syph.	Tuberc.	Gottier	
M. M.	101	4/20/14	24	♂	—	—	++	++	Lupus vulgaris?
F. K.	102	4/20/14	48	♂	—	—	++	++	Frontal and parietal headaches
I. H.	103	4/21/14	46	♂	—	—	++	++	Total blindness
Sch.	104	4/21/14	62	♂	—	—	++	++	Necrosis of toes
O. P.	105	4/21/14	46	♂	—	—	++	++	Aphasia on left side of face, occ. pain
D.	106	4/21/14	46	♂	—	—	++	++
A. C.	107	4/21/14	16	♂	—	—	++	++
G. G.	108	4/21/14	?	♀	—	—	++	++
M. F.	109	4/21/14	38	♀	—	—	++	++
M. L.	110	4/22/14	38	♀	—	—	++	++
McP.	111	4/22/14	39	♀	—	—	++	++
W. M.	112	4/22/14	31	♀	—	—	++	++
P. C.	113	4/22/14	36	♀	—	—	++	++
P. C.	114	4/22/14	62	♂	—	—	++	++
Sch.	115	4/23/14	40	♂	—	—	++	++
C. B.	116	4/24/14	22	♂	—	—	++	++
H.	117	4/24/14	24	♂	—	—	++	++
I. J.	118	4/29/14	34	♂	—	—	++	++
S. J.	119	4/29/14	27	♂	—	—	++	++
H. V.	120	4/29/14	24	♂	—	—	++	++
D. J.	121	4/29/14	35	♂	—	—	++	++
G. L.	122	4/29/14	40	♂	—	—	++	++
H. T.	123	4/30/14	47	♂	—	—	++	++
J. M.	124	4/30/14	18	♂	—	—	++	++
J. A. G.	125	4/30/14	18	♂	—	—	++	++
C. W. L.	126	4/30/14	3	♂	—	—	++	++
Ch.	127	5/1/14	35	♂	—	—	++	++
L. B.	128	5/1/14	57	♂	—	—	++	++
G. M.	129	5/1/14	25	♂	—	—	++	++
L. B.	130	5/1/14	30	♂	—	—	++	++
G. A. J.	131	5/1/14	19	♂	—	—	++	++
G. S.	132	5/2/14	25	♂	—	—	++	++
J. B.	133	5/2/14	8	♂	—	—	++	++
O.	134	5/2/14	28	♂	—	—	++	++
S. S.	135	5/2/14	25	♂	—	—	++	++
G. L.	136	5/2/14	35	♂	—	—	++	++
L. L.	137	5/2/14	31	♂	—	—	++	++
L. A. W.	138	5/2/14	..	♂	—	—	++	++
B.	139	5/2/14	..	♂	—	—	++	++
J. H.	140	5/2/14	30	♂	—	—	++	++
I. M. L.	141	5/2/14	30	♂	—	—	++	++
W. W.	142	5/2/14	21	♂	—	—	++	++
D. J.	143	5/2/14	20	♂	—	—	++	++

TABLE 2—SEROLOGIC FINDINGS IN 85 CASES WITH HISTORY OF SPECIFIC INFECTION*

Primary:							
1	3	10	11	16	17	18	24
++	0+	0+	++	++	++	++	00
32	54	120	132	155	172	179	
++	±+	00	++	00	0+	0+	
Secondary:							
7	15	21	45	56	59	62	63
++	++	++	++	++	++	++	++
68	80	89	96	91	97	99	100
++	++	++	00	++	00	++	++
119	130	153	162	186	193	199	
0+	++	++	++	++	0+	++	
Tertiary:							
12	28	40	50	64	65	69	75
++	++	++	++	0+	++	00	++
112	159						
++	++						
Latent:							
2	8	13	14	23	26	30	35
++	++	±±	++	++	++	++	++
37	41	57	92	98	127	131	144
++	++	++	++	++	0+	00	++
147	163	171	173	174	192		
++	++	++	0+	++	++		
Cerebral Lues:							
4	33	43	61	70	93		
++	++	++	++	Sp. f. +0	++		
Tabs:							
46	49	94	105	107	109	114	123
++	++	++	++	++	++	+0 Sp. f.	+0 Sp. f.
Congenital syphilis:							
95							
++							

* The designation to the left indicates the findings with the Wassermann reaction; to the right the result of the sero-enzyme test.

Table 3 gives the arrangement of these eighty-five cases according to the serologic findings.

TABLE 3.—ARRANGEMENT OF 85 CASES WITH HISTORY OF SPECIFIC INFECTION ACCORDING TO THE SEROLOGIC FINDINGS*

No. of Cases	Percentage	Wassermann Reaction	Sero-Enzyme Test
62	72.94	++	+
5	5.88	++	—
7	8.23	—	+
8	9.41	—	—
1	1.16	±	+
2	2.32	—	±

* In this table + = positive; — negative; ± doubtful.

Out of the five cases which gave a positive Wassermann reaction and a negative sero-enzyme for syphilis, three were cerebrospinal fluid which does not contain enzymes—Cases 70,

114, 123. The seven cases giving a negative Wassermann reaction and a positive sero-enzyme syphilis reaction were, 3 in the primary, 1 in the secondary, 1 in the tertiary, and 2 in the latent stages of syphilis. Case 54, primary chancre, gave a doubtful Wassermann reaction and a positive sero-enzyme. Cases 13 and 112, also primary chancre, gave a negative Wassermann reaction and a doubtful sero-enzyme reaction for syphilis.

TABLE 4.—ARRANGEMENT ACCORDING TO SEROLOGIC FINDINGS OF 115 CASES IN WHICH THE SPECIFIC HISTORY WAS NEGATIVE

Number	Percentage	Wassermann Reaction	Sero-Enzyme for Syphilis
31	26.95	+	+
23	20.00	+	—
13	11.3	—	+
44	38.26	—	—
4	3.47	+	±

Out of the 115 cases giving no history of specific infection, 24 presented clinical symptoms of the disease.

In summarizing the findings of Table 5 we find the Wassermann reaction positive and sero-enzyme reaction positive in 12 cases or 50 per cent; Wassermann reaction positive and sero-enzyme reaction negative in 3 cases or 12.5 per cent; Wassermann reaction negative and sero-enzyme reaction positive in 8 cases or 33.33 per cent; Wassermann reaction negative and sero-enzyme reaction negative in 1 case or 4.16 per cent.

The 3 cases giving a positive Wassermann reaction and a negative sero-enzyme syphilis reaction include 2 cases, that is, Cases 4 and 12, in which spinal fluid was tested. The cerebrospinal fluid does not contain the protective enzymes. The 8 cases giving a negative Wassermann reaction and a positive sero-enzyme syphilis reaction were: 2 cases of primary syphilis (Cases 66 and 195), 1 secondary (Case 197), 2 latent (Cases 19 and 25), 2 had syphilitic ulcers (Cases 38 and 194), and 1 case of tabes (Case 122). Aside from the 12 cases found to be positive to the Wassermann reaction and sero-enzyme test for syphilis in the series clinically syphilitic, 19 others were found among the remaining 91 whose serums reacted positive in both tests, or 16.52 per cent.

Of the 4 cases giving a positive Wassermann and a doubtful sero-enzyme reaction, Case 112 gave a single positive Wasser-

mann while the remaining 3 gave double positive results. These latter 3 patients may be suffering from specific infection, although no clue to it could be elicited in their history.

TABLE 5.—CASES IN WHICH SPECIFIC INFECTION WAS DENIED, DIAGNOSED CLINICALLY AS SYPHILIS

1	Alopecia areata, gastric disturb.....	58	+++	+
2	Alopecia areata	86	+	+
3	Aphasia, l, side of face.....	106	++	+
4	Blindness in right eye, Sp. fluid.....	113	+++	—
5	Cerebral lues	20	++	+
6	Gumma of testes	5	+++	+
7	Latent, husband known to be syph.	19	—	+
8	Latent, child syphilitic	25	—	+
9	Macroglossia	152	++	+
10	Necrosis of toes	104	++	+
11	Palmar syphilide	156	++	+
12	Paresis Sp. fluid	198	+++	—
13	Primary lesion	66	—	+
14	Primary lesion	184	—	—
15	Primary lesion	195	—	+
16	Retinitis	39	+++	—
17	Secondary syphilis	73	++	+
18	Secondary syphilis	101	++	+
19	Secondary syphilis	197	—	+
20	Syphilitic ulcers on legs	38	—	+
21	Syphilitic ulcers on legs	60	+++	+
22	Syphilitic ulcers in nose and throat.....	165	+++	+
23	Syphilitic ulcers on right tonsil	194	—	+
24	Tabes	122	—	+

TABLE 6.—WASSERMANN REACTION AND SERO-ENZYME TEST POSITIVE IN CASES IN WHICH SPECIFIC INFECTION WAS DENIED

		No.	Case
1	Anemia	1	71
2	Arthritis	1	141
3	Epilepsy	3	78, 196, 200
4	Epithelioma of tongue	1	44
5	Gonorrhea	3	87, 143, 178
6	Headache	2	6, 102
7	Mitral and aortic regurgitation	1	115
8	Pain in shoulder	1	77
9	Pelvic pain	1	96
10	Pleurisy	1	27
11	Tuberculosis	2	52, 136
12	Uncontrollable shaking of hands	1	9
13	Visceral Ptosis	1	53
19			

WASSERMANN REACTION POSITIVE, SERO-ENZYME DOUBTFUL

1	Blindness (total)	1	103
2	Coryza	1	76
3	Dizziness	1	111
4	Ulcerative condition of the toes	1	142
4			

The Wassermann reaction was positive and the sero-enzyme test for syphilis negative in 20 out of 115 cases, or 17.39 per cent. It is interesting to note that 9 cases of tuberculous condition in Table 7 gave a positive Wassermann reaction which, though faint, was distinct. Tuberculosis is capable of giving a positive Wassermann reaction. If we subtract the 9 cases of tuberculosis in Table 7 and Case 161, cancer of the throat, we have left 10 cases which possibly might be syphilitic.

TABLE 7.—WASSERMANN REACTION POSITIVE, SERO-ENZYME TEST NEGATIVE

		Case	No.
1	Asthma	1	160
2	Cancer of throat	1	161
3	Gonorrhea	1	190
4	Gon. rheumatism	1	180
5	Lupus vulgaris	1	22
6	Pain in arms and legs, elbows, wrist	3	74, 164, 189
7	Pain in left shoulder	1	148
8	Senile dementia	1	48
9	Tuberculosis	9	125, 128, 134, 138, 139, 140, 151, 176, 183
10	Wart under thumb-nail	1 20	117

Six serums of patients suffering from the conditions indicated in Table 8 gave a negative Wassermann reaction and a positive sero-enzyme test.

TABLE 8.—WASSERMANN REACTION NEGATIVE, SERO-ENZYME TEST POSITIVE

		Case	No.
1	Facial paralysis	1	175
2	Frozen foot refusing to heal	1	75
3	Gonorrhea	1	191
4	Mixed Infection (Gon.)	1	51
5	Run-down condition	1	169
6	Arthritis	1	67

Out of the remaining 42 cases, 36, or 39 per cent, were both negative for Wassermann reaction and the sero-enzyme test for syphilis. The clinical condition of these cases is given in Table 9.

TABLE 9.—SPECIFIC INFECTION DENIED. WASSERMANN REACTION AND SERO-ENZYME TEST NEGATIVE

	Case	No.
Acne rosacea	1	42
Black water fever	1	72
Burn Sec. Degree	1	124
Chancroid	1	31
Corvza	1	166
Diabetes	1	36
Epilepsy	2	34, 120
Exostosis on head	1	84
Gonorrhea	1	81
Goiter	3	116, 120, 177
Headache	2	29, 145
Injury to head	1	185
Normal	3	126, 158, 168
Ozena, erosion of nose	1	83
Pain in arm and legs	1	47
Pregnancy	1	146
Pleurisy	1	181
Run down condition	1	154
Saddle Nose	1	121
Sore throat	1	55
Stomatitis	1	170
Stomach trouble	1	182
Tuberculosis	11	85, 88, 108, 118, 133, 135, 137, 149, 150, 187, 188
Ulcers on 1. arm and fingers	1	82
Ulcer on forehead (Epithelioma?)	1	157
Warts on arm and hands	1	167

SUMMARY.

1. In conclusion I would point out that the sero-enzyme test for syphilis, when carried out with syphilitic testicular tissue, is probably a specific reaction, since the somatic cells are highly specialized, presupposing a specific enzyme for their cleavage. Thus, in the use of the gumma produced in rabbits by inoculation, we make use of a substratum which, when free from contamination, is superior to tissue prepared from the tissue of syphilitic patients, which of necessity is contaminated and is likely to give rise to positive reaction in other conditions.

2. The reaction is not applicable in cases in which cerebro-spinal fluid is to be tested.

3. The reaction is more specific than the Wassermann reaction, for it has always given a positive reaction with the serum of tabetics, while the Wassermann reaction in this condition is negative in about 40 per cent of cases. I would also point out the nine cases of tuberculosis which gave a positive Wassermann reaction although no sign of syphilis could be found in any

of these patients. The specificity of the test is further shown in the eight cases giving a negative Wassermann reaction and a positive sero-enzyme test (Summary of Table 5).

4. The technic of the reaction demands cleanliness and careful control of shells, tissue, serum, and the carrying out of the ninhydrin reaction. As one becomes acquainted with the test one's confidence in it increases.

5. The sero-enzyme test for syphilis represents, with the exceptions indicated, a true biologic test for this disease.

A CASE OF CONTAGIOUS BRONCHO-PNEUMONIA CAUSED BY *BACILLUS COLI COMMUNIS*.*

By EDWIN M. STANTON, DETROIT, MICH.

Hutyra and Marek¹ state that Enzootic Pneumonia of young animals is caused by the *Bacillus bipolaris septicus* or its varieties. However, cases are on record where the disease has been due to an infection with the *Bacillus pyogenes*, the *Streptococcus pyogenes*, and by the bacteria of the colon group. It is further stated that in other enzootic diseases, as white scour and dysentery of sucklings, contagious broncho-pneumonia occurs as a secondary infection. E. Wallis Hoare² says that broncho-pneumonia is caused by a variety of organisms; the bipolaris being the most common. He states that the *Bacillus coli communis* produces broncho-pneumonia as a secondary infection of white scour, and in the chapter upon white scour³ it is stated that the lung affection follows as a complication.

The statements of these two authors are exceedingly interesting. They show that it is possible for animals affected with a contagious intestinal disease to infect other animals with the organisms causing that intestinal disease, and that the latter animals may manifest entirely different symptom complex than that which existed in the animals primarily infected. The disease produced in the secondarily infected animals may involve a different anatomical system. In this way a secondary disease of a highly contagious nature is introduced into a herd. In other words, we have an organism such as *Bacterium coli communis*, that is capable of producing in the animal economy two highly contagious diseases of a different nature.

During November of 1913, a section of lung was sent to this laboratory by the late Doctor Frederick H. Osgood, of Boston, Mass., with the request that a diagnosis be made. The history accompanying the specimen stated that the specimen was from a six months old calf, which had died, exhibiting symptoms before

*Research Laboratory, Parke, Davis & Co.

death of a disease which had affected 40 other calves upon the premises, resulting in the death of these animals.

The symptoms as stated were as follows: Elevation of temperature, discharge from the mucus membranes of the eyes and nostrils, loss of appetite, emaciation and respiratory disturbances, and death. It was stated that the disease occurred after the introduction into the herd of some thoroughbred Jerseys.

Gross Pathology.—The surface of the lung tissue was greenish in color, roughened and covered with round and oval nodules about the size of a small pea. The nodules were soft, and when pressed a thick, creamy pus exuded. Upon cutting into the tissue the cut surface was of a dark red color. From the section of tissue, pus exuded. The tissue was heavier than water and non-crepitant.

Bacteriological Examination.—Smears from the pus were made and when stained revealed a few streptococci and numerous short bacilli.

Agar plates were made from the pus in the usual way. One set was incubated aerobically, the other anaerobically. After 24 hours incubation both series of plates contained many colonies of bacilli. No colonies of streptococci nor staphylococci were observed on either set of plates.

Subcultures were made upon plain agar slants and incubated 18 hours. Microscopical examination showed that the cultures consisted of a short bacillus. The organism was then transferred to differential media consisting of agar slant, plain agar deep, glucose agar deep, plain bouillon, plain and litmus milk, gelatin, potato and fermentation tubes of glucose, lactose and saccharose.

DESCRIPTION OF ORGANISM.—*Morphological characteristics.*—A short, plump motile rod, which is gram negative, and does not form spores.

Cultural Characteristics.—Plain agar, a translucent, raised, moist growth with wavy margins.

Deep Agar.—Translucent, confluent colonies along line of stab, and on the surface above the entrance of stab the growth raised and slightly grayish. A few gas bubbles observed in the body of the medium.

Glucose Agar Deep.—The growth the same as in agar deep. Medium, broken by gas formation.

Plain Bouillon.—Cloudy, viscid sediment formed at the bottom. A fecal odor was noticeable.

Plain Milk.—Coagulated, not digested. *Litmus Milk*.—Acid, coagulated. *Gelatin*.—Not liquified. Confluent colonies along line of puncture.

Potato.—Grayish white, glistening growth. *Glucose Bouillon*.—5 ccm. of gas, acid. *Lactose Bouillon*.—1 ccm. of gas, acid. *Saccharose Bouillon*.—3 ccm. of gas, faintly acid. *Durham's Peptone Solution*.—Indol formed.

From the cultural and morphological characters, the organism was identified as *Bacillus coli communis*.

From the pathological condition of the section of lung sent in, it was evident that a pneumonic condition existed. The symptoms stated pointed to a broncho-pneumonia, and from the bacteriological findings it appeared that the *Bacillus coli communis* was the causative agent.

REFERENCES.

1. Hutyra and Marek: "Pathology and Therapeutics of the Diseases of Domestic Animals." Vol. II., pages 134-135.
2. E. Wallis Hoare: "A System of Veterinary Medicine." Vol. I., page 496.
3. Ibid., page 504.

**REPRINTS OF PUBLICATIONS FROM THE RESEARCH
LABORATORY, PARKE, DAVIS & CO.,
DETROIT, MICH.**

The present system of collecting reprints of articles published from the Research Laboratory was begun in 1912. Reprints of the following articles published subsequent to that time are available and will be sent upon request. The publications marked (*) are no longer available.

1. On the Administration of Diphtheria Toxin in a Collodion Sac. By E. C. L. Miller. (*Journal of Infectious Diseases*, Vol. 8, January, 1911, pp. 50-65.)

2. A Further Contribution to Our Knowledge of Insecticides—Fumigants. By Chas. T. McClintock, H. C. Hamilton and F. B. Lowe. (*Journal of the American Public Health Association*, Vol. 1, April, 1911, pp. 227-238.)

3. Duboisia Hopwoodii—A Histological Study. By Oliver A. Farwell. (Reprinted from *Merck's Report*, Vol. 20, May 1, 1911.)

*4. Etiology of Canine Distemper. By Newell S. Ferry. (*Journal of Infectious Diseases*, Vol. 8, June, 1911, pp. 399-420.)

*5. The Resistance of Smallpox Vaccine to the Coal-tar Disinfectants. By Chas. T. McClintock and Newell S. Ferry. (*Journal of the American Public Health Association*, Vol. 1, June, 1911, pp. 418-419.)

6. Production of Immunity with Over-Neutralized Diphtheria Toxin. By Chas. T. McClintock and Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Originale, Bd. 59, July 15, 1911, pp. 456-464.)

7. Soaps from Different Glycerides—Their Germicidal and Insecticidal Values Alone and Associated with Active Agents. By H. C. Hamilton. (*Journal of Industrial and Engineering Chemistry*, Vol. 3, August, 1911, pp. 582-584.)

*8. The Sleepy Grass of New Mexico: A Histological Study. By Oliver A. Farwell. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-273.)

*9. Some Observations on the Physiological Action of Sleepy Grass. By A. W. Lescohier. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-275.)

*10. An Investigation of the Depressor Action of Pituitary Extracts. By Carey P. McCord. (*Archives of Internal Medicine*, Vol. 8, November, 1911, pp. 609-620.)

11. The Physiology of the Pituitary Gland and the Actions of Its Extracts. By Carl J. Wiggers. (*American Journal of Medical Sciences*, Vol. 141, April, 1911, pp. 502-515.)

12. A Physiological Investigation of the Treatment of Hemoptysis. By Carl J. Wiggers. (*Archives of Internal Medicine*, Vol. 8, 1911, pp. 17-38.)

13. Notes on Catgut Sterilization: A Preliminary Report. By Willard H. Hutchings. (*Annals of Surgery*, Vol. 54, July, 1911, pp. 693-695.)

14. The Relations of Pyogenic Microorganisms to the Etiology and Treatment of Skin Diseases. By Henry Rockwell Varney. (*Ohio State Medical Journal*, December, 1911.)

15. A Micrococcus with Unusual Characteristics as a Factor in a Resistant Dermatitis Resembling Acne Vulgaris. By Henry Rockwell Varney and L. T. Clark. (*Journal of Cutaneous Diseases*, Vol. 30, February, 1912, pp. 72-78.)

16. Serum Treatment of Hemorrhage and Blood Dyscrasias. By A. W. Lescohier. (*New York Medical Journal*, Vol. 95, February 3, 1912, pp. 223-229.)

*17. Further Studies on the Bacillus Bronchicanis, the Cause of Canine Distemper. By Newell S. Ferry. (*American Veterinary Review*, Vol. 41, April, 1912, pp. 77-79.)

18. The Pharmacopœial Requirements for Cannabis Sativa. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, March, 1912, pp. 200-203.)

19. The Heart Tonic Unit. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 84, March, 1912, pp. 97-103.)

20. Studies on the Etiology of Equine Influenza. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, April, 1912, pp. 185-197.)

21. A Method for the Bacteriological Standardization of Disinfectants. By Tatsuzo Ohno and H. C. Hamilton. (*American Journal of Public Health*, Vol. 2, May, 1912, pp. 331-338.)

22. Physiological Testing. By E. M. Houghton. (*American Drug-gist*, July and September, 1911, and January and April, 1912.)

23. Bacillus Bronchisepticus (Bronchicanis): The Cause of Distemper in Dogs and a Similar Disease in Other Animals. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, July, 1912, pp. 376-391.)

24. On Feeding Young Pups the Anterior Lobe of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 30, July, 1912, pp. 352-357.)

25. A Practical Portable Incubator. By Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Original, Bd. 65, Heft 4/5, 1912, pp. 412-413.)

26. Tobacco Extracts: Their Comparative Values as Insecticides. By W. O. Hollister. (*Journal of Economic Entomology*, Vol. 5, June, 1912, pp. 263-267.)

27. The Pharmacological Assay of Pituitary Preparations. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, October, 1912, pp. 1117-1119.)

28. Pituitary Extracts in Obstetrics and Gynecology. By A. W. Lescohier and O. E. Closson. (*Journal of the Michigan State Medical Society*, Vol. 11, October, 1912, pp. 650-657.)

29. Biological Products—Veterinary. By Robert H. Wilson. (*American Veterinary Review*, Vol. 41, September, 1912, pp. 668-681.)

30. The Isolation and Cultural Characteristics of Bacillus Aene. By Edwin M. Stanton. (*Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Original, Bd. 66, Heft 5/7, 1912, pp. 386-389.)

31. Studies on Hog Cholera. By Walter E. King and Robert H. Wilson. (*Journal of Infectious Diseases*, Vol. 11, Nov., 1912, pp. 441-458.)

32. Studies on the Virus of Hog Cholera. By Walter E. King and F. W. Baeslark. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 39-41.)

33. The Physiological Activity of Cannabis Sativa. By H. C. Hamilton, A. W. Lescohier and R. A. Perkins. (*Journal of the American Pharmaceutical Association*, Vol. 2, Jan., 1913, pp. 22-30.)

34. The Iodine Content of the Small, Medium and Large Thyroid Glands of Sheep, Beef and Hogs. By T. B. Aldrich. (Original Communications, Eighth International Congress of Applied Chemistry, Vol. 19, 1912, pp. 9-14.)

35. Studies on the Virus of Hog Cholera. By Walter E. King and Robert H. Wilson. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 16, Heft 3, 1913, pp. 367-376.)

36. On the Cultivation of the Treponema Pallidum (Spirochæta Pallida). By F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 55-67.)

*37. Studies on the Gonococcus, I. By Carl C. Warden. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 93-105.)

38. Studies on the Virus of Hog Cholera. By Walter E. King, F. W. Baeslack and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 12, March, 1913, pp. 206-235.)

39. Bacillus Bronchisepticus—Its Relation to Canine Distemper. By N. S. Ferry. (*American Veterinary Review*, Vol. 43, April, 1913, pp. 16-30.)

40. Drug Influence on Extrasystoles of the Mammalian Heart. By Carey P. McCord. (*Interstate Medical Journal*, Vol. 19, Oct., 1912, pp. 870-880.)

41. The Employment of Protective Enzymes of the Blood as a Means of Extracorporeal Diagnosis. I.—Sero-Diagnosis of Pregnancy. By Carey P. McCord. (*Surgery, Gynecology and Obstetrics*, Vol. 16, April, 1913, pp. 418-421.)

42. Tribromo-tert-Butyl Alcohol, $C_4H_9OBr_3$. By T. B. Aldrich. (*Journal of the American Chemical Society*, Vol. 33, March, 1911, pp. 386-388.)

43. On Feeding Young White Rats the Posterior and the Anterior Parts of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 31, Nov., 1912, pp. 94-101.)

44. The Rationale of the Use of Adrenalin in the Treatment of Asthma. By Carey P. McCord. (*Medical Record*, Vol. 83, March 8, 1913, pp. 431-432.)

45. Standardization of Disinfectants: Some Suggested Modifications. By H. C. Hamilton and T. Ohno. (*American Journal of Public Health* Vol. 3, June, 1913, pp. 582-588.)

46. Preventive Measures Against Equine Influenza Based on Its Bacteriology. By N. S. Ferry. (Report of the Proceedings of the United States Live Stock Association, December, 1912, p. 127.)

47. Correcting Water. By H. C. Hamilton. (*Bulletin of Pharmacy*, Vol. 27, August, 1913, pp. 330-335.)

48. Duration of Immunity Following Small-pox Vaccination. By A. W. Leschier. (*Journal of the American Medical Association*, Vol. 61, Aug. 16, 1913, page 487-490.)

49. On Crystalline Kombe-Strophanthin. By D. H. Brauns and O. E. Closson. (*Journal of the American Pharmaceutical Association*, May, June and July, 1913, Vol. 2.)

50. A Comparative Study of Antigens for the Wassermann Reaction. By H. R. Varney and F. W. Baeslack. (*Journal of the American Medical Association*, Vol. 61, Sept. 6, 1913, pp. 754-757.)

51. The Treatment of Tetanus. By Charles T. McClintock and Willard H. Hutchings. (*Journal of Infectious Diseases*, Vol. 13, Sept., 1913, pp. 309-320.)

52. Spirochæta Suis, Its Significance as a Pathogenic Organism, Studies on Hog Chlorea. By Walter E. King and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 13, Nov., 1913, pp. 463-498.)

53. Time Recorder for Kymograph Tracings. By Oliver E. Closson. (*Journal of Pharmacology and Experimental Medicine*, Vol. 5, Jan., 1914, pp. 235-238.)

54. U. S. P. Menstrua. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 86, Feb., 1914, pp. 56-61.)

55. Numerical Variations of the White Blood Cells in Mice Inoculated with Transplantable Adenocarcinoma. By F. W. Baeslack. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 20, Heft 5, 1914, pp. 421-435.)

56. A Study of the Germicidal Action of the Ultraviolet Rays. By E. M. Houghton and L. Davis. (*American Journal of Public Health*, Vol. 4, March, 1914, pp. 224-240.)

57. Some Phenomena Involved in the Life History of *Spirochæta Suis*—Studies on Hog Cholera. By W. E. King and R. H. Drake. (*The Journal of Infectious Diseases*, Vol. 14, March, 1914, pp. 246-250.)

58. The Sterilization of Adrenalin Solutions. By L. W. Rowe. (*American Journal of Pharmacy*, Vol. 86, April, 1914, pp. 145-149.)

59. Infection and Immunity: A Review. By N. S. Ferry, Ph.B., M.D. (*Journal of the American Pharmaceutical Association*, Vol. 3, April and May, 1914.)

60. Disinfection—What Disinfectant is the Most Generally Applicable for Clinical, Surgical and Sanitary Purposes? By H. C. Hamilton. (*Therapeutic Gazette*, Vol. 38, May, 1914, pp. 311-315.)

61. Study of the Bacteriology of the Posterior Nasopharynx in Scarlatina. By N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, pp. 934-935.)

62. Some Experiences with Bacterial Vaccines in Scarlatina. By Guy L. Kiefer, M.D., D.P.H., and N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, p. 936.)

63. A Sero-enzyme Test for Syphilis. By F. W. Baeslack, M.A., M.D. (*The Urologic and Cutaneous Review*, Vol. 18, May, 1914, pp. 234-238.)

64. Bacteriology and Control of Acute Infections in Laboratory Animals. By N. S. Ferry, Ph.B., M.D. (*Journal of Pathology and Bacteriology*, Vol. 18, 1914, pp. 445-455.)

65. The Bacteriological Standardization of Disinfectants. By H. C. Hamilton and Tatsuzo Ohno. (*American Journal of Public Health*, Vol. 4, No. 6, p. 163.)

66. The Pineal Gland in Relation to Somatic, Sexual and Mental Development. By Carey P. McCord, M.D. (*Journal of the American Medical Association*, July 18, 1914, Vol. 63, pp. 232-235.)

67. The Sero-enzyme Test for Syphilis. By F. W. Baeslack, M.D., M.A. (*Journal of the American Medical Association*, Aug. 15, 1914, Vol. 63, pp. 559-563.)

68. A Case of Contagious Broncho-pneumonia Caused by *Bacillus Coli Communis*. By Edwin M. Stanton. (*American Veterinary Review*, Vol. 14, May, 1914, pp. 233-235.)

LOCAL ANESTHETICS — SOME COMPARATIVE PHYSIOLOGICAL REACTIONS.

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The search for cocaine substitutes has brought out many local anesthetics of decided merit and, for certain uses, preferable to cocaine. In ophthalmic work or the anesthesia of unbroken mucous surfaces, cocaine is still the local anesthetic generally used. According to Buckley, it has scarcely a rival in any field; others (1) extol some other of the local anesthetics. For par-enteral injection, there is little uniformity of opinion as to the best local anesthetic—in fact this is dependent upon the results desired, long or short anesthesia, rapidity of action, after period of numbness, etc. If it is desired to block impulses along nerves or to act as an ordinary local anesthetic largely on the nerve ending, then different anesthetics may be desired, as also when some of the side reactions as contraction of the capillaries, absence of vasoconstrictor power, or the interference with the hemostatic action of adrenalin (2) must be considered.

A great many investigators (3) have already contributed to this question, but in view of the conflicting results, Dr. E. M. Houghton has suggested that the more meritorious local anesthetics be re-examined if only for corroboratory purposes.

The large mass of clinical data which has accumulated upon local anesthetics is of little help in understanding the value of the various preparations, as it is always aimed to employ doses well within the safety zone, and as the degree of anesthesia above a certain minimum is indeterminate. It is in fact found that much smaller doses are required than was at first supposed necessary. But this minimum dose or concentration is always exceeded, so that it affords but an uncertain basis for studying comparative relations. Clinicians look carefully at the toxicity of a preparation, yet a failure to appreciate the significance of the toxicity of a preparation is met with on every side. It is not the relative toxicity but toxicity relative to the desired activity or

safety factor, not only with local anesthetics but with other drugs as well, that should be considered. Quinine and urea hydrochloride is stated to be nontoxic (4). Toxicity tests on guinea-pigs show, however, that cocaine hydrochloride is only about six times as toxic as quinine and urea hydrochloride, yet there is no hesitancy in employing a 10-per-cent. solution to do the work of a 1-per-cent. solution of cocaine hydrochloride. According to test, such a solution of quinine is nearly twice as dangerous as cocaine. To be sure this is based on the toxicity to guinea-pigs, and may not be so dangerous for man, but in any event it should not be looked upon as absolutely safe even if familiarity with quinine has bred contempt. Certainly a 50-per-cent. quinine and urea hydrochloride solution should be used with due regard to the volume injected.

As a basis for comparison, the absolute values of the toxic and irritating properties of some local anesthetics when injected subcutaneously into guinea-pigs were determined and are recorded in Table I. These *per se* have no value, but in comparison with their anesthetic power are or should be the principal determining factor in the acceptance or rejection of a local anesthetic.

Considering the properties recorded in Table I, they take the following order as regards desirability:

Novocaine, beta eucaine, quinine salts, tropacocaine, stovaine, cocaine and alypin.

The irritant properties of alypin place it last, notwithstanding its being slightly less toxic for the guinea-pig than cocaine, also quinine may be considered less desirable than tropa-cocaine on account of its irritant action, and the quinine and urea hydrochloride less desirable than quinine hydrochloride. The other substances in the table are not strictly local anesthetics although they act as such on the motor nerve of a frog.

METHOD OF COMPARING ANESTHETIC ACTION.

Anesthetic action was compared in two ways, by the degree and duration of anesthesia produced in the conjunctiva of a rabbit by dropping the solutions in the eye, and by the character of the action of the solution upon the nerve muscle preparation

TABLE I.
Toxicity to Guinea Pigs When Injected Subcutaneously.

MATERIAL	Dose in g. per kg.	RESULTS		SYMPTOMS	LESIONS AT SITE	REMARKS
		Lived	Died			
Mercuric chloride 1 in 1000 water solution.	0.005	4	0	Fur rough, list- less, weak.	Congestion, infil- tration appears necrotic.	Death in 3 to 4 days.
	*0.008	0	2			
	0.01	0	3			
	0.02	0	5			
Cocaine hydro- chloride 1 in 50 and 1 in 100 water solution	0.03	3	1	Excitable, spas- modic convul- sions Death thru failure resp.	Inflammation, very slight.	Death in 20 min.
	0.04	5	1			
	*0.05	2	6			
	0.06	0	3			
Alypin (hydro- chloride) 1 in 100 water solution.	0.03	1	0	Excitable same picture as with cocaine. Death by failure resp.	Inflammation marked.	Death in 1 hour.
	0.04	3	0			
	0.05	3	1			
	*0.06	2	4			
	0.07	0	3			
Stovaine (hydro- chloride 1 in 20 water solution.	0.1	3	0	Similar to co- caine; very rapid running move- ments. Death by failure resp.	Marked conges- tion.	Death in 20 min.
	0.15	2	0			
	*0.2	0	4			
	0.3	0	4			
Tropacocaine hy- drochloride 1 in 20 water solution.	0.1	3	1	Convulsive twitch- ings much like cocaine. Death by failure resp	Inflamed.	Death in 20 min.
	0.15	3	1			
	*0.2	1	2			
	0.3	0	1			
Chloretone and Urethane equal parts form a liquid sp. gr. 1.22.	0.15	1	0	Prostrate com- pletely anaesthe- tized, fur rough.	Large infiltration with congestion.	Death in about 24 hrs.
	0.20	3	1			
	0.30	1	4			
	0.40	0	1			
	0.50	0	2			
Quinine mono- chloride, 1 in 40 water solu- tion.	0.1	1	0	Excited inco-ordi- nation, spasmo- dic convulsions.	Inflamed not as large infiltration as with the urea salt.	Death in about 24 hrs.
	0.2	4	1			
	*0.3	2	3			
	0.4	0	3			
Quinine and urea hydrochlor- ide 1 in 10 wa- ter solution.	0.1	2	0	Spasmodic convulsions.	Inflamed area ap- pears necrotic.	Death in 15 to 30 hrs.
	0.2	7	3			
	0.3	7	7			
	*0.4	1	9			
	0.5	1	6			
Beta Eucaine 1 in 40 water solution.	0.1	1	0	Convulsive twitchings	Congested, ap- pears necrotic.	Death in 1 to 3 days.
	0.25	1	0			
	0.50	3	1			
	*0.40	0	3			
	0.5	0	2			
Novocaine hydro- chloride 1 in 20 water solution.	0.2	2	0	Convulsive twitchings	Slight inflamma- tion.	Death in 1 to 24 hrs.
	0.3	3	0			
	*0.4	0	5			
	0.5	0	1			
Chloretone in olive oil 1 in 10 and 1 in 20	0.25	1	0	Prostrate, com- pletely anaesthe- tized.	Considerable in- flammation.	Death in 3 to 48 hrs.
	0.3	4	1			
	*0.4	1	3			
	0.5	0	5			
	0.7	1	5			
Brometone in olive oil 1 in 20.	0.3	3	0	Prostrate, com- pletely anaesthe- tized.	Inflammation marked.	Death in 5 to 36 hrs.
	*0.4	0	2			
	0.5	0	2			
	0.6	0	1			
	0.7	1	5			
Phenol 1 in 100 water solution.	0.2	1	0	Tremors and twitching.	Inflamed slightly.	Death within 24 hours.
	0.4	2	0			
	0.5	5	1			
	*0.6	1	5			
	0.7	1	5			
	0.8	0	2			
Para-phenyl-sulphonic salt of ethyl para- amino-benzoate, 1 in 50 water solution	0.6	1	0	Prostrate, no con- vulsive strug- gles.	Small infiltration, no inflammation.	Death within 2 days.
	0.7	4	1			
	*1.0	1	4			
	1.5	0	2			
	1.0	2	0			
Ethyl-para- amino-benzoate in olive oil 1 in 20.	1.1	2	0	Prostrate no struggles.	Infiltration, no inflammation.	Death in 24 hours.
	*1.2	1	2			
	1.3	0	2			
	1.5	0	1			
	1.0	4	1			
Potassium sul- phate 1 in 25 and 1 in 50.	1.5	4	3	Drowsy no strug- gles or hyper- sensitiveness.	Infiltration rather inflamed and necrotic with the large doses.	Death in 24 to 72 hrs.
	*1.8	0	2			
	2.0	2	4			
	2.3	0	2			
	2.3	0	2			

*Minimum Lethal dose.

‡A very small amount of hydrochloric acid was sufficient to bring into solution which was practically neutral to litmus, no trace of acidity.

TABLE II.
Table of Ophthalmic Experiments With Rabbits.

MATERIAL INSTILLED INTO EYE	per cent. sol.	Irritability Control eye	Recorded in Cm. at Which Secondary Coil just stimulated.										REMARKS
			Treated Eye		After Interval of:								
			0 min.	5 min.	10 min.	15 min.	20 min.	30 min.	45 min.	60 min.			
Cocaine hydrochloride water solution	2 5 5 10	21 23 23.5 22	21 23 23.5 23.5	20 21 20.5 22	20.5 21.0	22	21.5 23 21.5 22.5	23 23.5 23.5	20 23		Pale, then slight inflammation, no hypersecretion.		
Stovaine water solution	5 5	22.5 23.5	23 23	19 22	23	20.5	23.5	20.5 23.5	23.5		Inflamed, hypersecretion.		
Tropacocaine hydrochloride water solution	5 5	22 22.5	22 23	21	20.5	19.5	21.5	20.5 22.5	21.5 23		Very slight inflammation and hypersecretion.		
Alcaph hydrochloride water solution	1 3 5	20.5 21 24.5	21 21 24.5	20.5 23.5	21 24	20 24	21 25.5	21 27	21 25	21, 2 hrs.	Vessels injected. Persistent inflammation		
Beta Eucaine hydrochloride water solution	4	23.5	23	22.5	22.5	23	23.5	23.5	23.5		Slight inflammation, hypersecretion.		
Novocaine hydrochloride water sol.	5	27	27	27		26			25	28, 1½ hr.	Very slight inflammation, no hypersecretion.		
Paraphenyl sulfonic salt of ethyl p. aminobenzoate water sol.	2	22	22	24		23			22		No inflammation, very slight hypersecretion.		
Quinine and Urea hydrochloride water sol.	10 10 5	20 22 23.5	20 22 23.5	23.5	23.5	20	18 21 23	16 21 22.5	17 20 12.5	20, 2 hrs. 23.5, 2 hrs.	Marked congestion and hypersecretion.		
Quinine hydrochloride water sol.*	5	21	21		18	10		18	18	17, 2 hrs.	Marked congestion, but less than above, and hypersecretion.		
Olive oil		23	23	21.5		21.5			22	21.5	22, 2 hrs.	Normal.	
Castor oil		23	23	20.5		21.5			21.5	21.0	22, 2 hrs.	Normal.	
Chloretone in castor oil	20 20	19 23	19 23	21	15	22		16	22			Congestion.	
Ethyl para aminobenzoate in castor oil	2½ 5	21 22	21 22.5	20 21			19.5 21		21 21.5	21, 4 hrs.		Very slight inflammation.	
Ethyl para aminobenzoate in olive oil	5	23.5	23.5	22	23		22.5		22.5	21.5, 2 hrs. 21.0, 4 hrs.			

*The monochloride with just sufficient hydrochloric acid to bring into solution (reaction neutral to litmus.)

of the frog. This data on the ophthalmic method is collected in Table II.

The ophthalmic method while more comparable with the practical requirements, in that it is dealing with sensory nerves, on the other hand requires the penetration of unbroken mucous surfaces.

The degree of anesthesia produced in the conjunctiva was determined by the amount of electric current necessary to

cause a reflex movement of the eyelid, always using the opposite eye as a control. The stimulating current was applied to the conjunctiva close to the edge of the lid using blunt platinum electrodes three millimeters apart.

The source of the current was an induction coil with 300 turns on the primary, of one ohm resistance and 13,000 turns on the secondary of 2,550 ohms resistance. The regular lighting circuit D. C. 110 volt was led through a sixteen candle power, carbon filament lamp to the primary coil and ordinary magnetic make and break mechanism. By inserting a thirty-two candle power lamp as a shunt, around the apparatus, all burning of the platinum contacts was avoided, the maximum potential difference being reduced to forty volts and the minimum to 0.25 volts. As the resistance of the primary with leads was one ohm the maximum current was 0.25 ampere. With the circuit breaker in action twenty-eight to twenty-nine volts were recorded depending on the position of the secondary.

The least current that could be detected when the electrodes were placed on the tip of the tongue was that given with the secondary coil twenty-five centimeters distant from the primary. In all experiments stimulation was applied by opening a short circuit in the secondary for a fraction of a second only.

The results of this ophthalmic method are very unsatisfactory (5) for determining anesthetic power, as can be observed by consulting Table II. The small change in current is a little surprising as mechanical stimulation often will not show any signs of sensation. The value of oil solutions is in general uncertain as oil alone necessitates a greater current for stimulation. However, the strong chloretone solution is undoubtedly markedly anesthetic, but the irritation of such a strong solution nullifies its practical value.

Of the water soluble anesthetics cocaine and tropacocaine are powerful and but slightly irritating; alypin, stovaine, and beta eucaine are fairly efficient anesthetics but are more irritating; quinine salts, while distinctly anesthetic, are very irritating in strengths 3% or over exhibiting anesthetic power to the unbroken mucous membrane. Novocaine and ethyl p. amino-benzoate while non-irritating possess but weak anesthetic power.

It would appear that none of these examined are as efficient

as cocaine for anesthesia of unbroken mucous surfaces; however, in ophthalmic work, the dilation of the pupil may be a disadvantage and one of the next four be preferred (6).

The effect on motor nerves and nerve endings, while not absolutely comparable with sensory nerves and nerve endings, gives, nevertheless, exact data for comparative relations which clinically ($\hat{\gamma}$) are also found to hold for the sensory nerves.

In some qualitative comparisons on the effect upon the sciatic nerve and gastrocnemius muscle of frogs I failed to obtain the same results as reported by L  wen (3).

The whole series was, therefore, examined, using the Leopard frog, *Rana pipiens*, and to be comparable, also, the large *Rana catibian* from New Orleans.

The animals were decapitated, skinned and the sciatic nerve carefully dissected out as far as the gastrocnemius muscle, which was left intact. The central end of the nerve was tied and cut off just before it enters the pelvic girdle, and stimulation was applied only to this smooth nerve trunk. This part of the nerve between the thread and the muscle was laid in loops made in the end of six platinum wires which acted as electrodes. These loops or hooks were arranged five millimeters apart, the first electrode coming as near the muscle as possible, and the nerve was stimulated across each of these intervals. That nearest the muscle is designated A, then follow B, C, D and E; the E being nearest the central end of the nerve. For convenience of manipulation an apparatus much like that described by L  wen (3) was used, the electrodes being fixed to a hinged piece so that the nerve could be easily raised and lowered. This allows stimulation at the same point each time without handling. To further facilitate the readings, a two-armed switch was fitted to the board so that any two adjacent electrodes could be immediately connected for stimulation.

The current given by a secondary coil as well as the available stimulating current is dependent upon many factors, as has been very well pointed out by Martin (15); however, without going into such details cognizance may be taken of the fact that for practical purposes the secondary current varies inversely as the square of the distance. As the secondary is brought closer to

the primary this relation is less exact but still gives more comparable relations than linear comparisons.

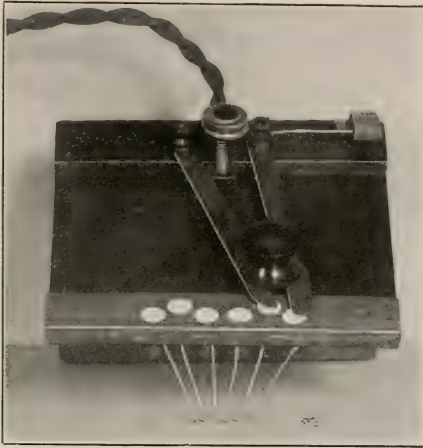


Fig. 1. Apparatus for applying stimulation.

Stimulation was applied by means of the induction coil the same as with the conjunctiva experiments. However, in the following protocols and curves, instead of recording the maximum distance at which the secondary coil would deliver a stimulating current the inverse square of the distance is recorded in terms of units, the unit being the current given by this particular coil connected as described when the secondary is 100 centimeters from the primary. The units = $(100/X)^2$, X being the distance of the secondary from the primary. For instance, if $X = 25$ centimeters then $(100/25)^2 = 16$ units.

The conductivity changes in the nerve are apparent from comparison of the minimum stimulating current at A, B, C, D and E, and the irritability by the magnitude of minimum stimulating currents. The direct stimulation of the muscle is recorded as M and allows a differentiation of the effect on nerve ending and on the muscle tissue.

Typical of a number of experiments where *Rana pipiens* 20 to 30 g. were used are the results seen in Table III. Even one-tenth of this strength gives no recovery in over an hour, as seen in Table IV. L  wen, however, left preparations in 5-per-cent. cocaine hydrochloride for an hour without extinction of the irritability.

TABLE III.
5% Cocaine Hydrochloride.

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	2.4	2.4	2.5	2.5	2.5	16	Normal
	Replaced by 5% Cocaine Hydrochloride.						
5 min.	Nothing at 100					35	
5 min.	Replaced by Ringer's solution						
15 min.	Nothing at 100					50	
30 min.	Nothing at 100					50	
120 min.	Replaced by fresh Ringer's solution						
120 min.	Nothing at 100					80	
7 hrs.	Nothing at 100					35	

TABLE IV.
1/2% Cocaine Hydrochloride.

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	2.5	2.4	2.5	3.0	2.7		Normal
	Replaced by 50 cc 1/2% (a) Cocaine Hydrochloride						
5 min.	5	5	6	12	5		
15 min.	Nothing at 100						
15 min.	Replaced by Ringer's sol.						
25 min.	Nothing at 100						
35 min.	Nothing at 100						
70 min.	Nothing at 100						

That the *Rana pipiens* are not especially susceptible is seen in V and VI, where the large frogs from New Orleans were used.

TABLE V.
5% Cocaine Hydrochloride.
Frog of 530 G.

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	6	6	6	5.5	5	15	Normal
	Replaced by 200 cc 5% Cocaine Hydrochloride.						
5 min.	9	6	6	9	7	25	
15 min.	Nothing at 100						
15 min.	Replaced by Ringer's solution						
25 min.	Nothing at 100						35
40 min.	Nothing at 100						25
55 min.	Nothing at 100						30
115 min.	Nothing at 100						40

The control leg had remained unchanged and was used in VI.

TABLE VI.
1/10% Cocaine Hydrochloride.
Control Leg of V. Frog of 530 G.

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	5	6	6	6	6	18	Normal
	Replaced by 1/10% (a) Cocaine Hydrochloride.						
15 min.	7	12	9	9	9	35	
25 min.	70	100	90	90	90	40	
25 min.	Replaced by Ringer's solution						
40 min.	70	100	100	100	100	37	
75 min.	50	100	90	90	70	30	
95 min.	30	70	50	50	35	30	
125 min.	25	70	40	25	25	30	

The recovery with the nerve muscle preparation of the large frog is seen to be slower than with that of the small frogs of VII, as would be expected from physical considerations.

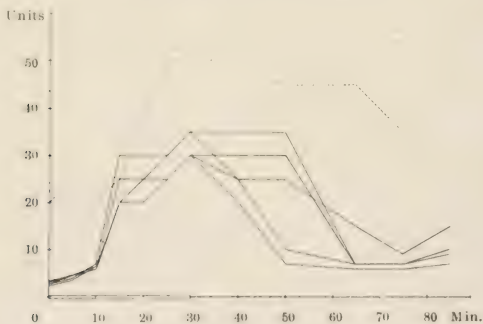
The plotting of the time as abscissas and units as ordinates gives a much clearer idea of the character of reaction than the tables of figures, and has been done with the representative tables for the different anesthetics.

The graph VII shows a number of points in connection with the specific action of cocaine on the nerve elements. The most obvious is that the effect on nerve endings is more rapid than on the nerve fibre, so that during the immersion the action on the nerve fibre is masked, but appears when placed in the Ringer's solution. The recovery from the effect on the nerve endings is even more rapid than the recovery of

TABLE VII.
1/10% Cocaine Hydrochloride.

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	3.2	3.4	2.7	2.7	2.5	20	Normal
Replaced by 1/10% (a) Cocaine Hydrochloride.							
5 min.	4.5	4.5	4.5	4.0	3.6	25	
10 min.	6	6.5	6.5	7.0	6.5	20	
15 min.	20	20	20	30	25	25	
20 min.	20	20	25	30	25		
25 min.	25	25	25	30	30	50	
30 min.	30	30	30	35	35		
30 min.	Replaced by Ringer's solution						
40 min.	20	25	30	35	25	50	
50 min.	7	10	30	35	25	45	
65 min.	6	7	7	7	15	45	
75 min.	6	7	7	7	9	35	
85 min.	7	9	9	10	15	25	

the fibre, so that the decreased conductivity of the nerve fibre is distinctly seen. I believe Mosso & Frank (*Arch. de. Physiol.*, 1892, p. 562) first observed such an affinity between cocaine and nerve tissue.



VII.

A more specific affinity is seen with tropacocaine VIII, where the action on the muscle tissue is slight, so that the masking of the lowered conductivity is surely due to an action on nerve endings.

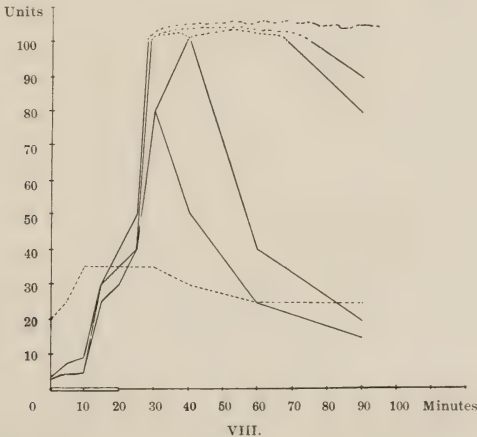
The action of tropacocaine is also rapid and energetic, and although more closely related to cocaine in chemical structure than beta eucaine, yet in physiologic response beta eucaine is closer to cocaine. Apparently tropacocaine is nearly as active as cocaine, while beta eucaine is easily seen to be weaker. (IX.)

TABLE VIII.
1/10% Tropacocaine Hydrochloride.

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	3.4	3.4	2.7	3.0	3.0	20	Normal
Replaced by 50 cc 1/10% (a) Tropacocaine Hydrochloride.							
5 min.	3.8	3.4	3.8	3.4	7.0	25	
10 min.	4.5	4.5	4.5	4.5	9.0	35	
15 min.	25	30	30	30	30	35	
20 min.	30	35	35	35	40	35	
20 min.	Replaced by Ringer's solution						
25 min.	40	40	40	40	50	35	
30 min.	80	100	80	100	100	35	
40 min.	50	100	100	100	100	30	
60 min.	25	40	100	100	100	25	
60 min.	Replaced by fresh Ringer's solution.						
90 min.	15	20	80	90	100	25	

It takes longer to produce its effects, but when once established (X) the recovery is slow.

Novocaine being the least active of the anesthetics so far recorded, the effect of a 5-per-cent. solution might be expected to give the results



reported by Læwen; however, as is seen in the table the result is the same as noted with cocaine.

After one minute the 5-per-cent. novocaine solution has completely blocked the nerve impulse, and upon replacing by Ringer's solution after five minutes, no recovery is shown in over two hours.

TABLE IX.
1/10% Beta Eucaïne Hydrochloride.

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	1.6	1.8	1.7	2.7	2.2		Normal
Replaced by 50 cc 1/10% Beta Eucaïne Hydrochloride.							
5 min.	3.0	3.5	7.0	12	8		
10 min.	3.5	8.0	12.0	16.0	14		
15 min.	20	30	30	35	25		
15 min.	Replaced by 50 cc Ringer's solution						
25 min.	35	35	40	35	30		
35 min.	30	25	30	30	30		
35 min.	Replaced by fresh Ringer's solution						
60 min.	7	12	20	45	20		

TABLE X.
1/10% Beta Eucaïne Hydrochloride.

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	3.0	3.0	3.0	2.7	2.2	20	Normal
Replaced by 50 cc 1/10% (a) Beta Eucaïne Hydrochloride.							
5 min.	3.4	3.6	3.4	4.5	5.0	30	
10 min.	3.4	4.0	3.6	4.5	5.0		
15 min.	6.5	6.5	7.0	7.0	8.0	45	
20 min.	18	20	20	20	25	45	
20 min.	Replaced by Ringer's solution						
25 min.	15	18	20	20	25	45	
35 min.	20	25	25	25	30	55	
45 min.	25	18	20	20	25	60	
60 min.	25	20	25	30	25	60	
80 min.	20	18	18	20	20	50	

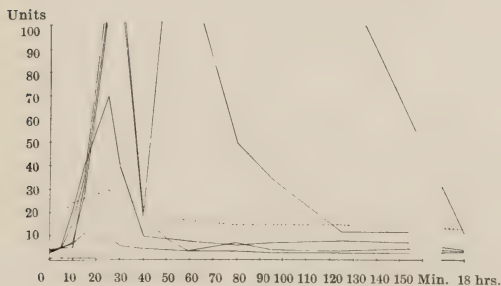


TABLE XI.
5% Novocaine Hydrochloride.

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	2.9	2.8	2.9	3.8	1.8		Normal
	Replaced by 5% Novocaine Hydrochloride						
5 min.	100	100	100	100	100		No response after one min.
5 min.	Replace by Ringer's solution						
25 min.	100	100	100	100	100		
30 min.	Replaced by fresh Ringer's solution						
55 min.	100	100	100	100	100	60	
	Replaced by fresh Ringer's solution						
2 hrs.	100	100	100	100	100	55	

TABLE XII.
2/10% Novocaine Hydrochloride.

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	3.2	4.0	2.5	2.4	2.7	15	Normal
	Replaced by 50 cc 2/10% (a) Novocaine Hydrochloride.						
5 min.	5	5	4.5	5	4.5		
10 min.	6.5	20	5.0	7.0	15	24	
15 min.	11	40	30	27	35		
20 min.	Changed to Ringer's solution						
25 min.	10	70	100	100	100	30	
30 min.	6	40	100	100	100		
40 min.	4.5	10	18	20	100		
50 min.	4.0	9	9	100	100	18	
60 min.	3.5	3.5	8.0	100	100		
80 min.	3.2	7.0	6.0	50	100	15	
95 min.	2.7	4.0	7.0	35	100		
125 min.	2.7	3.5	8.0	12	100	15	
155 min.	2.7	4.5	7.0	12	100		
18 hrs.	3.0	3.5	4.0	11	11	13	



XII.

When a 2/10-per-cent. solution is used, a picture very comparable with that of the other anesthetics is given.

It is distinctly seen in XII that an attack on nerve conductivity is the principal mode of action, so that novocaine should be very useful in the nerve blocking type of local anesthesia.

Quite different is the action of the closely related body anesthesin, which acts very much like phenol.

The paraphenyl sulphonic acid salt of anesthesin (ethylester of para-amino benzoic acid) is fairly soluble and shows an action largely upon the muscle and nerve endings. The character of the response is similar to that with phenol, showing a nearly constant plane of anesthesia with a given strength.

The equilibrium plane of anesthesia is clearly shown in tables XVI and XVII.

TABLE XIII.

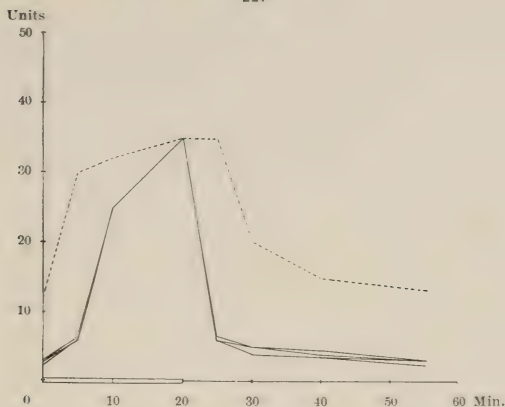
Paraphenylsulphonate of Ethyl P. Aminobenzoate.

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	3.0	2.9	3.0	2.7	2.7	12	Normal
	Replaced by 50 cc 5/100% (a) of above						
5 min.	5.0	5.0	4.5	7.0	5.0		
15 min.	6.0	6.0	6.0	6.0	7.0	22	
35 min.	6.5	6.5	6.0	6.5	7.0	30	
35 min.	Replaced by Ringer's solution						
45 min.	4.0	4.0	4.0	4.0	4.5	15	
55 min.	3.4	3.7	3.4	4.0	4.5	15	
75 min.	3.0	3.0	3.0	3.4	3.0	12	

TABLE XIV.

1/10% Paraphenylsulphonate of Ethyl P. Aminobenzoate.

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	2.7	3.0	3.0	2.7	2.2	12	Normal
	Replaced by 50 cc 1/10% (a) of above.						
5 min.	6.0	6.5	6.5	6.5	6.0	30	
10 min.	25	25	25	25	25	32	
15 min.	30	30	30	35	30	32	
20 min.	35	35	35	35	35	35	
20 min.	Replaced by Ringer's solution						
25 min.	6.0	6.0	6.0	6.5	6.0	35	
30 min.	4.0	5.0	5.0	5.0	4.0	30	
40 min.	3.4	4.0	4.0	4.5	3.4	15	
55 min.	3.0	3.0	3.0	3.0	2.3	13	



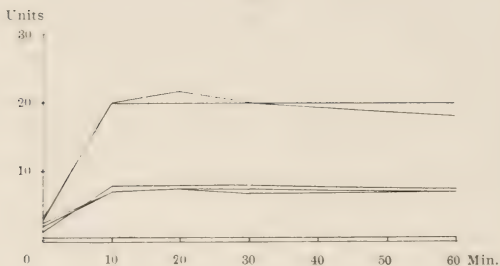
XIV.

TABLE XV.
2/10% Paraphenylsulphonate of Ethyl P. Aminobenzoate.

Time Interval		Minimum Stimulating Current in Units						Remarks
		A	B	C	D	E	M	
		2.3	2.2	2.1	2.1	1.8	12	Normal
		Replaced by 50 cc 2/10% (a) of above						
5	min.	80	100	100	100	100	27	
7	min.	Replaced by Ringer's solution						
10	min.	60	100	100	100	100		
15	min.	60	80	80	80	90	100	
20	min.	13	45	23	30	40		
25	min.	16	11	16	15	21	70	
30	min.	16	16	14	14	14		
40	min.	4.5	14	10	9	11	11	
100	min.	4.5	9	10	9	10	12	
160	min.	3.2	6.5	11	10	13	17	

TABLE XVI.
1/10% Phenol.

Time Interval		Minimum Stimulating Current in Units.						Remarks
		A	B	C	D	E	M	
		2.4	2.1	1.9	3.0	2.7		Normal
		Replaced by 50 cc 1/10% (a) Phenol.						
10	min.	7	8	7	20	20		
20	min.	7.5	8	7.5	22	20		
30	min.	7	8	7.5	20	20		
60	min.	7	7.5	7.0	20	18		

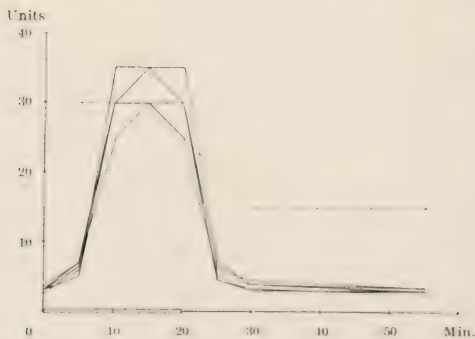


XVI.

TABLE XVII.

2/10% Phenol.

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	3.0	3.4	3.0	3.0	2.5	15	Normal
	Replaced by 50 cc 2/10% (a) Phenol						
5 min.	5.0	7.0	7	6.5	6.0	30	
10 min.	30	30	25	30	35	30	
15 min.	30	35	30	35	35	30	
20 min.	30	30	25	30	35		
20 min.	Replaced by Ringer's solution						
25 min.	4.5	4.5	5.0	6.0	70	30	
35 min.	3.4	3.0	4.0	4.5	4.0	15	
45 min.	2.7	3.0	3.4	3.4	3.0	15	

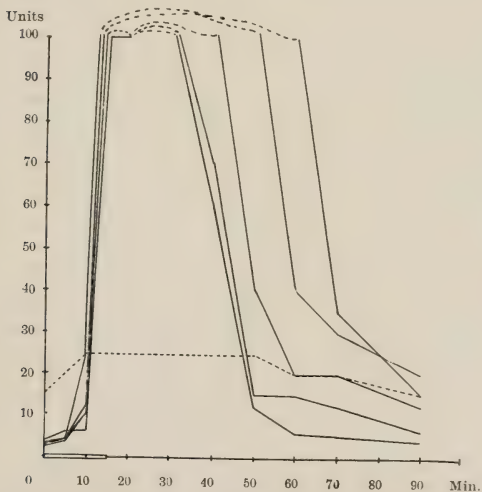


XVII.

Stovaine shows a marked interference with nerve conduction, but very little action on the threshold irritability of the muscle tissue. The

TABLE XVIII.
1/10% Stovaine.

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	3.8	3.4	3.0	2.5	25	15	Normal
	Replaced by 50 cc 1/10% (a) Stovaine						
5 min.	5.5	4.0	4.0	3.4	3.0	20	
10 min.	1.0	10	12.0	10	25.0	25	
15 min.	100	100	100	100	100	25	
15 min.	Replaced by Ringer's solution						
20 min.	100	100	100	100	100		
30 min.	None	at 100				25	
40 min.	60	70	100	100	100	25	
50 min.	12	15	40	100	100	25	
60 min.	6	15	20	100	40	20	
70 min.	5	12	20	35	30	20	
90 min.	4.5	6.0	12	15	20	15	



XVIII.

response to stimulation was, however, very small in magnitude, even with considerable increase in current.

Alypin shows a greater reduction of muscle irritability than was ex-

TABLE XIX.
1/10% Alypin.

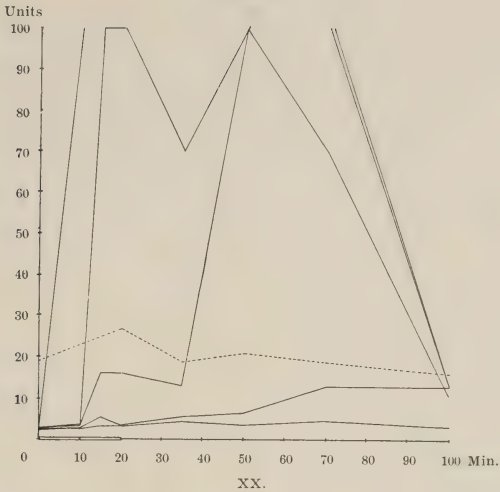
Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	2.3	2.6	2.2	3.0	2.4	21	Normal
	Placed in 50 cc 1/10% (a) Alypin.						
5 min.	3.2	3.0	3.5	3.0	3.0	35	
10 min.	3.5	4.0	3.2	4.0	4.5	35	
15 min.	4.5	5.0	4.0	5.0	4.5	35	
17 min.	Replaced by Ringer's solution						
20 min.	5.0	6.0	5.0	5.5	5.0	30	Responses shallow
							even at 100
30 min.	5.5	6.5	8.0	5.5	11.0	25	Responses only a quiver at 100
40 min.	6.0	100	100	100	100	20	
50 min.	100	100	100	100	100	17	
80 min.	100	100	100	100	100	19	
120 min.	100	100	100	100	100	16	
180 min.	100	100	100	100	100	19	
16½ hrs.	100	100	100	100	100	20	

hibited by stovaine, and also an interference with nerve conduction; however, the action on the nerve shows the difficultly reversible effect suggestive of quinine salts. The peculiar rigid condition of the muscle with toes distended was also seen, which seems to be significant of quinine, and bodies which show the properties of a general protoplasmic poison.

Even with very weak solutions, alypin shows its affinity for nerve and muscle tissue.

TABLE XX.
2/100% Alypin.

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	2.2	2.1	2.4	2.4	2.7	19	Normal
	Replaced by 2/100% (a) Alypin						
10 min.	2.4	2.6	3.0	3.5	100		
15 min.	3.0	5.0	16	100	100		Toes distended, leg slightly rigid
20 min.	3.0	3.2	16	100	100	27	
	Replaced by Ringer's solution						
35 min.	4.5	5.5	13	70	100	19	
50 min.	3.5	6.5	100	100	100	21	
70 min.	4.5	13	70	100	100	19	
100 min.	3.3	13	11	13	13	16	



Quinine salts attack the muscle tissue primarily, the muscle becoming rigid, the toes distended. An effect upon conductivity is also indicated in Table XI.

TABLE XXI.
2/10% Quinine and Urea Hydrochloride.

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	2.8	3.0	2.6	2.8	3.4	14	Normal
Replaced by 2/10% (a) Quinine and Urea Hydrochloride.							
5 min.	2.8	3.0	2.4	2.8	3.4	21	Toes distended
10 min.	3.4	3.0	2.5	3.2	4.0	30	
15 min.	2.9	2.8	2.7	4.0	5.0		
20 min.	3.0	3.0	2.7	3.7	6.0	35	
20 min. Replaced by Ringer's solution							
25 min.	2.9	2.5	2.6	4.0	6.0	25	
35 min.	2.6	2.1	3.2	3.2	6.0	18	
45 min.	2.6	2.4	3.1	3.1	3.3	16	
70 min.	2.7	2.8	2.8	3.3	3.7	15	

With a stronger solution, Table XXII, the conductivity is seen to be markedly affected, but with no action on the nerve ending.

The recovery of the irritability of the nerve was generally slower than

TABLE XXII.
3/10% Quinine and Urea Hydrochloride.

Time Interval		Minimum Stimulating Current in Units						Remarks
		A	B	C	D	E	M	
		2.2	2.5	2.9	3.5	4.0	11	Normal
		Replaced by 3/10% (a) Solution.						
5	min.	2.2	2.8	15	70	100	16	Toes dis- tended
10	min.	3.7	17	100	100	100	27	Muscle rigid
10	min.	Replaced by Ringer's solution						
20	min.	2.2	4.0	35	100	100	14	
30	min.	2.2	3.7	35	100	100	14	
45	min.	2.5	3.2	13	100	100	13	Muscle re- laxed
70	min.	3.0	4.0	6.0	100	100	12	
100	min.	2.9	2.4	2.3	21	100	12	
140	min.	2.9	2.6	2.8	3.4	9.0	11	
185	min.	2.7	3.0	2.8	3.0	4.5	11	

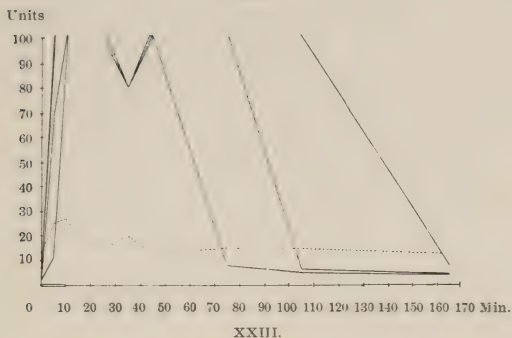
appears here, and often the nerve appeared to be injured so that it only partially recovered and then regressed, eventually losing all irritability.

Quinine monochloride shows the same action as the double salt; with urea hydrochloride it is, however, more powerful, apparently about 4/3 as active, which from the per cent. of quinine would be expected.

TABLE XXIII.
3/10% Quinine Monochloride.

Time Interval		Minimum Stimulating Current in Units						Remarks
		A	B	C	D	E	M	
		2.4	2.5	2.6	2.9	3.7	13	Normal
		Replaced by 50 cc 3/10% (a) Quinine Monochloride.						
5	min.	11	70	100	100	100	25	Toes dis- tended mus- cle rigid
10	min.	100	100	100	100	100	27	
10	min.	Replaced by Ringer's solution						
15	min.	100	100	100	100	100	20	
25	min.	100	100	100	100	100	15	
35	min.	100	80	80	80	100	20	
45	min.	100	100	100	100	100	13	
75	min.	8.0	100	100	100	100	15	Muscle re- laxing
105	min.	5.0	6.5	100	100	100	15	
165	min.	4.0	4.5	8.0	100	100	13	

The local anesthetics take the following order as regards anesthetic power on the nerve muscle preparation of the frog: alypin,



cocaine, stovaine, tropacocaine, beta eucaine, novocaine, quinine salts, paraphenyl sulphonate of ethyl p. aminobenzoate, and phenol.

These, however, act in different ways, so that for nerve blocking, alypin, stovaine, novocaine and quinine salts come first; while for nerve endings cocaine, tropacocaine, beta eucaine, stovaine, paraphenyl sulphonate of ethyl p. aminobenzoate and phenol are more efficient in the order given.

In stronger solutions the action of these is not necessarily proportional to the concentration. Schmid (8) has shown that the combination of two local anesthetics does not give an increased action, and from his work it appears also that the anesthetic action is below the mean where two are combined; except beta eucaine with novocaine and cocaine with tropacocaine.

The anesthetic action of some of the local anesthetics can be increased by salts with weak (9) acidic radicals and by the use of potassium (10) salts.

It was thought that possibly Læwen used merely sodium chloride as an indifferent fluid, and that possibly the small amount of potassium chloride in Ringer's solutions would account for the more pronounced action observed with *Rana pipiens* and the large swamp frog of New Orleans.

It was found that using 75/100 per cent. (11) sodium chloride in place of Ringer's for diluting, a quite different picture was obtained.

TABLE XXIV.

2/10% Cocaine Hydrochloride.

- A. By diluting with Ringer's solution.
 B. By diluting with 75/100% sodium chloride.

Time Interval	LEFT LEG			RIGHT LEG		
	Minimum Current	Stimulating Current	in Units	Minimum Current	Stimulating Current	in Units
	A	B	M	A	B	M
Normal	4.0	3.4	14	3.4	2.8	17
	Placed in A			Placed in B		
5 min.	4.5	4.0	16	3.5	3.3	19
10 min.	4.5	4.5	19	4.0	3.7	27
15 min.	19	25	23	4.5	3.7	25
20 min.	21	35	25	4.5	3.7	25
20 min.	Changed to Ringer's			Changed to 75/100 NaCl.		
30 min.	25	80	32	4.5	5.5	21
40 min.	19	80	25	4.5	4.0	23
50 min.	21	50	27	4.5	3.3	19
50 min.	Changed to Ringer's			Changed to Ringer's		
60 min.	8.0	60	27	4.0	3.3	17
65 min.				4.0	11	30
70 min.	6.5	60	25	4.5	19	30
75 min.	Changed to Solution A.			Changed to Solution A.		
85 min.	4.5	23	23	6.5	25	30
85 min.	Changed to Ringer's			Changed to Ringer's		
95 min.	4.5	13	21	11	45	30
105 min.	4.5	6.5	17	4.5	80	30
125 min.	4.5	4.0	15	4.5	7.0	25
155 min.	4.5	3.7	16	4.5	3.3	23

However, as no salts were used in the earlier experiments, an attempt was made to duplicate Læwen's results by the use of a hypertonic solu-

TABLE XXV.

3 1/3% Cocaine Hydrochloride.

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	3.7	5.5	4.0	3.4	3.5	23	Normal
	Placed in 3 1/3% Cocaine Hydrochloride Solution.						
min.	100	100	100	100	100	70	
5 min.	Replaced by 75/100% Sodium Chloride.						
10 min.	Nothing at 100						50
20 min.	Nothing at 100						45
30 min.	Nothing at 100						45
40 min.	Nothing at 100						27
50 min.	Nothing at 100						30
80 min.	Nothing at 100						25

tion, a 10-per-cent. solution of cocaine hydrochloride being diluted with an equal volume of 75/100-per-cent. sodium chloride. The same extinction quickly resulted, however.

A $3\frac{1}{3}$ -per-cent. cocaine solution, made by diluting a 10-per-cent. water solution with an equal volume of water and the same volume of 75/100-per-cent. sodium chloride, shows the following effect.

It thus appears that the apparently augmentative effect of potassium salts on the anesthetic by no means accounts for the discrepancy.

A 2/10-per-cent. potassium sulphate solution is seen to be prompt and marked in its action.

TABLE XXVI.

2/10% Potassium sulphate (by diluting a 1.3% water solution with 75/100% sodium chloride).

Time Interval	Minimum Stimulating Current in Units						Remarks
	A	B	C	D	E	M	
	2.1	2.7	3.3	3.3	3.1	15	Normal
	Placed in above solution.						
5 min.	2.1	2.6	3.3	3.2	3.3	16	
15 min.	12	40	45	50	60	19	
	Replaced by 75/100% Sodium Chloride.						
25 min.	2.5	2.7	2.7	3.8	6.5	13	
35 min.	2.4	3.1	3.7	3.7	4.0	12	

It thus seems that at least a part of the augmentative effect of potassium salts is the additive action of the anesthetic potassium sulphate, rather than the multiplication (10) of the action of cocaine, novocaine, etc., which has been assumed.

It is seen that the action of potassium salts is upon the nerve conductivity. A concentration of potassium salts is Nature's own way of deadening pain (12), for when a nerve is injured there is a concentration of potassium salts in the nerve trunk near the injury, so that stimulation must be very intense to pass through the area of the nerve in which there is found an increase of potassium salts.

The subdural injection of 2/10 cc. of a solution (1) containing $\frac{1}{2}$ -per-cent. potassium sulphate and 67/100-per-cent. sodium chloride, and of a solution (2) containing 9/10-per-cent. sodium chloride, into two persons, A.W.L. and O.E.C., showed with the first solution a very appreciable numbness for some time, while sodium chloride alone gave just after the injection a doubtful effect. However, when a 1/100-per-cent. cocaine solution, made by diluting a 2-per-cent. cocaine (Codrenin A) with the first solution, was injected, a very distinct anesthesia was produced, persisting over fifteen minutes.

There is thus an apparent enhancing action of potassium salts on cocaine over the simple additive action, although in this case the action must be largely on nerve endings, while potassium acts primarily on the nerve trunks.

From clinical data which will appear in a later communication, it appears that very dilute cocaine solutions with potassium sulphate are perfectly reliable. A 1/20 per cent. cocaine hydrochloride and 1/2-per-cent. potassium sulphate solution, with which we have more data, seems to be as efficient as a 1-per-cent. cocaine hydrochloride solution. While in the majority of cases there is no need of using as strong a solution as 1 per cent., it also appears that considerably below 1/20-per-cent. cocaine hydrochloride when combined with potassium sulphate is perfectly satisfactory.

To attain the dose of one-half grain of cocaine hydrochloride set by the U.S.P., it would be necessary to inject 80 cc., nearly three ounces, of this 1/20-per-cent. solution.

The order of anesthetic power as determined on the nerve muscle preparation of the frog appears to be: alypin, cocaine, stovaine, tropacocaine, beta eucaine, novocaine, quinine salts, anesthesin, phenol.

CONCLUSION.

In drawing conclusions from experiments upon the motor nerves and nerve endings of the frog, it is well to bear in mind that the function of a local anesthetic is to deaden sensory nerve endings, and block impulses along sensory nerves. Dixon (13) has shown that cocaine blocks impulses along sensory nerves better than along motor, so that the clinical results with sensory nerves may well show greater divergence of action than is generally assumed (7).

In general these results show that:

1. Cocaine is efficient in weaker solution than generally employed, and for prompt action coupled with the same relative safety has no rival.
2. Novocaine while less toxic is also weaker in anesthetic power than cocaine, alypin, stovaine, tropacocaine or beta eucaine.
3. Beta eucaine, while more toxic and more irritating than novocaine, is in the weak solutions necessary for local anesthesia, safer than novocaine and more prompt in its action.

4. Quinine, while slightly weaker than novocaine, shows a longer anesthesia, so that for nerve blocking (14) and post-surgical anesthesia, it is to be preferred.

5. Potassium salts are themselves local anesthetics, but augment the action of other anesthetics to a greater extent than the mere additive increase.

REFERENCES.

1. Bier, *Deut. Z. f. Chirurgie*, XCV, Nos. 1-5, considers tropacocaine the safest and cocaine the most dangerous for spinal anesthesia, and employs tropacocaine with adrenalin (cf. ref. 2).
- J. Oebler, *Beit. Z. klin. Chirurgie*, LV, No. 1, in Kummel's service, also considers tropacocaine the least dangerous spinal anesthetic.
- Stovaine was at first highly extolled and is still used extensively in spinal anesthesia. W. W. Babcock, *J.A.M.A.*, 61, p. 1353.
- Novocaine is at present in favor, H. Braun, *Deut. med. Woch.*, 31, p. 1667, first demonstrating its action. Quinine salts are, however, advancing to popularity. cf. A. E. Hertzler. Operations with local anesthesia; also series of articles in *Am. Jr. Surgery*, 1910 and 1911.
2. Stovaine partly destroys and tropacocaine almost completely destroys the vasoconstrictor power of adrenalin. Novocaine, however, increases the vasoconstrictor power of adrenalin. F. R. Smyth, *Proc. Roy. Soc. of Med.*, Vol. 4, p. 56.
- Novocaine appears most suited to cataphoresis. M. Lungren. A. Schéle and B. Svedin. *Zentr. Biochem. Biophys.*, 15, p. 119.
- Influence of cocaine on accommodation. I. Horovitz, *Inaug. Diss.*, 1912; *Zentr. Biochem. Biophys.*, 15, p. 94, also 14, p. 316.
- Upon intraocular tension, cocaine exerts sometimes an increase and sometimes a decrease. A. Fourriere, *Ann. Oculist*, 15, p. 95.
3. Impens. *Pflüger's Arch.*, 110, p. 21.
- A. Læwen. *Arch. Path. u. Pharm.*, 56, p. 188. *Beit. z. klin. Chirurgie*, 50, p. 621.
- H. Braun. *Deut. Z. f. Chirurgie*, 1911, p. 321.
- Lokalanesthetie, 3rd, 1913.
- Le Brocq. *Brit. Med. Jr.*, 1909, 1, p. 783.
- W. L. Symes and V. H. Veley. *Proc. Roy. Soc. B.*, Vol. 83, p. 421.
4. From the fact that Brewster used 100 grains intravenously in six hours and concludes it absolutely safe, *Jr. A.M.A.*, 53, p. 1395, L. J. Hirschman, *Proctologist*, 47, p. 122, concludes "It is absolutely nontoxic."
5. J. Morganroth and S. Ginsberg. *Berliner klin. Woch.*, 49, p. 2183. *Ibid.*, 50, p. 343, using as a standard the time of suppression of sensation to mechanical stimulation obtained, however, very comparable data.
6. F. M. Fernandez, *Therap. Gazette*, 26, p. 10, strongly advocates Alypin in ophthalmic work.
7. A. Læwen. *Münch. med. Woch.*, 57, p. 2044.
8. A. Schmid. *Zeit. Exp. Path. u. Therap.* 14, p. 527, cf.; also L. Horn, *ibid.*, 12, p. 529.
9. O. Gros. *Münch. med. Woch.*, 57, p. 2042.
- A. P. Mathews. *Am. J. Physiol.*, 11, p. 454.
10. H. Braun. *Zeit. f. Chirurgie*, 40, p. 1513.
- A. Hoffman, *ibid.*, No. 35.
- A. Hoffman, and M. Kochmann. *Deut. med. Woch.*, 38, p. 2264.
11. Commercial sodium chloride, the C. P. is quite toxic, deadening the muscle and nerve.
12. M. L. Menton. *Am. Jr. Physiol.*, 31, p. 85.
- MacDonald. *Proc. Royal Soc. B.*, 76, p. 332.
13. W. E. Dixon. *Jr. Physiol.*, 32, p. 87.
14. M. L. Harris. *Jr. Am. Med. Ass.*, 41, p. 1042.
15. E. G. Martin. *Am. Jr. Physiol.*
- Vol. 22, p. 61 to 74 and 116 to 132.
- Vol. 24, p. 269 to 285.
- Vol. 26, p. 181 to 190.
- Vol. 27, p. 226 to 239.
- Vol. 28, p. 49 to 56.
- (a) In all experiments where the anesthetic is used in strength below 5 per cent., it is made by diluting the 5-per-cent. water solution with Ringer's solution, and when insoluble to the extent of 5 per cent. the substance was placed in a container and dissolved in Ringer's solution to which enough water was added to correspond to a 5-per-cent. water solution of the substance used.

REPRINTS OF PUBLICATIONS FROM THE RESEARCH LABORATORY, PARKE, DAVIS & CO., DETROIT, MICH.

The present system of collecting reprints of articles published from the Research Laboratory was begun in 1912. Reprints of the following articles published subsequent to that time are available and will be sent upon request. The publications marked (*) are no longer available.

1. On the Administration of Diphtheria Toxin in a Collodion Sac. By E. C. L. Miller. (*Journal of Infectious Diseases*, Vol. 8, January, 1911, pp. 50-65.)

2. A Further Contribution to Our Knowledge of Insecticides—Fumigants. By Chas. T. McClintock, H. C. Hamilton and F. B. Lowe. (*Journal of the American Public Health Association*, Vol. 1, April, 1911, pp. 227-238.)

3. Duboisia Hopwoodii—A Histological Study. By Oliver A. Farwell. (Reprinted from *Merck's Report*, Vol. 20, May 1, 1911.)

*4. Etiology of Canine Distemper. By Newell S. Ferry. (*Journal of Infectious Diseases*, Vol. 8, June, 1911, pp. 399-420.)

*5. The Resistance of Smallpox Vaccine to the Coal-tar Disinfectants. By Chas. T. McClintock and Newell S. Ferry. (*Journal of the American Public Health Association*, Vol. 1, June, 1911, pp. 418-419.)

6. Production of Immunity with Over-Neutralized Diphtheria Toxin. By Chas. T. McClintock and Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Originale, Bd. 59, July 15, 1911, pp. 456-464.)

7. Soaps from Different Glycerides—Their Germicidal and Insecticidal Values Alone and Associated with Active Agents. By H. C. Hamilton. (*Journal of Industrial and Engineering Chemistry*, Vol. 3, August, 1911, pp. 582-584.)

*8. The Sleepy Grass of New Mexico: A Histological Study. By Oliver A. Farwell. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-273.)

*9. Some Observations on the Physiological Action of Sleepy Grass. By A. W. Lescohier. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-275.)

*10. An Investigation of the Depressor Action of Pituitary Extracts. By Carey P. McCord. (*Archives of Internal Medicine*, Vol. 8, November, 1911, pp. 609-620.)

11. The Physiology of the Pituitary Gland and the Actions of Its Extracts. By Carl J. Wiggers. (*American Journal of Medical Sciences*, Vol. 141, April, 1911, pp. 502-515.)

12. A Physiological Investigation of the Treatment of Hemoptysis. By Carl J. Wiggers. (*Archives of Internal Medicine*, Vol. 8, 1911, pp. 17-38.)

13. Notes on Catgut Sterilization: A Preliminary Report. By Willard H. Hutchings. (*Annals of Surgery*, Vol. 54, July, 1911, pp. 693-695.)

14. The Relations of Pyogenic Microorganisms to the Etiology and Treatment of Skin Diseases. By Henry Rockwell Varney. (*Ohio State Medical Journal*, December, 1911.)

15. A Micrococcus with Unusual Characteristics as a Factor in a Resistant Dermatitis Resembling *Aene Vulgaris*. By Henry Rockwell Varney and L. T. Clark. (*Journal of Cutaneous Diseases*, Vol. 30, February, 1912, pp. 72-78.)

16. Serum Treatment of Hemorrhage and Blood Dyscrasias. By A. W. Lescohier. (*New York Medical Journal*, Vol. 95, February 3, 1912, pp. 223-229.)
- *17. Further Studies on the Bacillus Bronchicanis, the Cause of Canine Distemper. By Newell S. Ferry. (*American Veterinary Review*, Vol. 41, April, 1912, pp. 77-79.)
18. The Pharmacopœial Requirements for Cannabis Sativa. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, March, 1912, pp. 200-203.)
19. The Heart Tonic Unit. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 84, March, 1912, pp. 97-103.)
20. Studies on the Etiology of Equine Influenza. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, April, 1912, pp. 185-197.)
21. A Method for the Bacteriological Standardization of Disinfectants. By Tatsuzo Ohno and H. C. Hamilton. (*American Journal of Public Health*, Vol. 2, May, 1912, pp. 331-338.)
22. Physiological Testing. By E. M. Houghton. (*American Druggist*, July and September, 1911, and January and April, 1912.)
23. Bacillus Bronchisepticus (Bronchicanis): The Cause of Distemper in Dogs and a Similar Disease in Other Animals. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, July, 1912, pp. 376-391.)
24. On Feeding Young Pups the Anterior Lobe of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 30, July, 1912, pp. 352-357.)
25. A Practical Portable Incubator. By Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Original, Bd. 65, Heft 4/5, 1912, pp. 412-413.)
26. Tobacco Extracts: Their Comparative Values as Insecticides. By W. O. Hollister. (*Journal of Economic Entomology*, Vol. 5, June, 1912, pp. 263-267.)
27. The Pharmacological Assay of Pituitary Preparations. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, October, 1912, pp. 1117-1119.)
28. Pituitary Extracts in Obstetrics and Gynecology. By A. W. Lescohier and O. E. Closson. (*Journal of the Michigan State Medical Society*, Vol. 11, October, 1912, pp. 650-657.)
29. Biological Products—Veterinary. By Robert H. Wilson. (*American Veterinary Review*, Vol. 41, September, 1912, pp. 668-681.)
30. The Isolation and Cultural Characteristics of Bacillus Acne. By Edwin M. Stanton. (*Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Original, Bd. 66, Heft 5/7, 1912, pp. 386-389.)
31. Studies on Hog Cholera. By Walter E. King and Robert H. Wilson. (*Journal of Infectious Diseases*, Vol. 11, Nov., 1912, pp. 441-458.)
32. Studies on the Virus of Hog Cholera. By Walter E. King and F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 39-41.)
33. The Physiological Activity of Cannabis Sativa. By H. C. Hamilton, A. W. Lescohier and R. A. Perkins. (*Journal of the American Pharmaceutical Association*, Vol. 2, Jan., 1913, pp. 22-30.)
34. The Iodine Content of the Small, Medium and Large Thyroid Glands of Sheep, Beef and Hogs. By T. B. Aldrich. (Original Communications, Eighth International Congress of Applied Chemistry, Vol. 19, 1912, pp. 9-14.)

35. Studies on the Virus of Hog Cholera. By Walter E. King and Robert H. Wilson. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 16, Heft 3, 1913, pp. 367-376.)

36. On the Cultivation of the Treponema Pallidum (Spirochaeta Pallida). By F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 55-67.)

*37. Studies on the Gonococcus, I. By Carl C. Warden. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 93-105.)

38. Studies on the Virus of Hog Cholera. By Walter E. King, F. W. Baeslack and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 12, March, 1913, pp. 206-235.)

39. Bacillus Bronchisepticus—Its Relation to Canine Distemper. By N. S. Ferry. (*American Veterinary Review*, Vol. 43, April, 1913, pp. 16-30.)

40. Drug Influence on Extrasystoles of the Mammalian Heart. By Carey P. McCord. (*Interstate Medical Journal*, Vol. 19, Oct., 1912, pp. 870-880.)

41. The Employment of Protective Enzymes of the Blood as a Means of Extracorporeal Diagnosis. I.—Sero-Diagnosis of Pregnancy. By Carey P. McCord. (*Surgery, Gynecology and Obstetrics*, Vol. 16, April, 1913, pp. 418-421.)

42. Tribromo-tert-Butyl Alcohol, $C_4H_9OBr_3$. By T. B. Aldrich. (*Journal of the American Chemical Society*, Vol. 33, March, 1911, pp. 386-388.)

43. On Feeding Young White Rats the Posterior and the Anterior Parts of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 31, Nov., 1912, pp. 94-101.)

44. The Rationale of the Use of Adrenalin in the Treatment of Asthma. By Carey P. McCord. (*Medical Record*, Vol. 83, March 8, 1913, pp. 431-432.)

45. Standardization of Disinfectants: Some Suggested Modifications. By H. C. Hamilton and T. Ohno. (*American Journal of Public Health*, Vol. 3, June, 1913, pp. 582-588.)

46. Preventive Measures Against Equine Influenza Based on Its Bacteriology. By N. S. Ferry. (Report of the Proceedings of the United States Live Stock Association, December, 1912, p. 127.)

47. Correcting Water. By H. C. Hamilton. (*Bulletin of Pharmacy*, Vol. 27, August, 1913, pp. 330-335.)

48. Duration of Immunity Following Small-pox Vaccination. By A. W. Lescohier. (*Journal of the American Medical Association*, Vol. 61, Aug. 16, 1913, page 487-490.)

49. On Crystalline Kombe-Strophanthin. By D. H. Brauns and O. E. Closson. (*Journal of the American Pharmaceutical Association*, May, June and July, 1913, Vol. 2.)

50. A Comparative Study of Antigens for the Wassermann Reaction. By H. R. Varney and F. W. Baeslack. (*Journal of the American Medical Association*, Vol. 61, Sept. 6, 1913, pp. 754-757.)

51. The Treatment of Tetanus. By Charles T. McClintock and Willard H. Hutchings. (*Journal of Infectious Diseases*, Vol. 13, Sept., 1913, pp. 309-320.)

52. Spirochaeta Suis, Its Significance as a Pathogenic Organism. Studies on Hog Cholera. By Walter E. King and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 13, Nov., 1913, pp. 463-498.)

53. Time Recorder for Kymograph Tracings. By Oliver E. Closson (*Journal of Pharmacology and Experimental Medicine*, Vol. 5, Jan., 1914, pp. 235-238.)

54. U. S. P. Menstrua. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 86, Feb., 1914, pp. 56-61.)

55. Numerical Variations of the White Blood Cells in Mice Inoculated with Transplantable Adenocarcinoma. By F. W. Baeslack. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 20, Heft 5, 1914, pp. 421-435.)

56. A Study of the Germicidal Action of the Ultraviolet Rays. By E. M. Houghton and L. Davis. (*American Journal of Public Health*, Vol. 4, March, 1914, pp. 224-240.)

57. Some Phenomena Involved in the Life History of Spirochæta Suis—Studies on Hog Cholera. By W. E. King and R. H. Drake. (*The Journal of Infectious Diseases*, Vol. 14, March, 1914, pp. 246-250.)

58. The Sterilization of Adrenalin Solutions. By L. W. Rowe. (*American Journal of Pharmacy*, Vol. 86, April, 1914, pp. 145-149.)

59. Infection and Immunity: A Review. By N. S. Ferry, Ph.B., M.D. (*Journal of the American Pharmaceutical Association*, Vol. 3, April and May, 1914.)

60. Disinfection—What Disinfectant is the Most Generally Applicable for Clinical, Surgical and Sanitary Purposes? By H. C. Hamilton. (*Therapeutic Gazette*, Vol. 38, May, 1914, pp. 311-315.)

61. Study of the Bacteriology of the Posterior Nasopharynx in Scarlatina. By N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, pp. 934-935.)

62. Some Experiences with Bacterial Vaccines in Scarlatina. By Guy L. Kiefer, M.D., D.P.H., and N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, p. 936.)

63. A Sero-enzyme Test for Syphilis. By F. W. Baeslack, M.A., M.D. (*The Urologic and Cutaneous Review*, Vol. 18, May, 1914, pp. 234-238.)

64. Bacteriology and Control of Acute Infections in Laboratory Animals. By N. S. Ferry, Ph.B., M.D. (*Journal of Pathology and Bacteriology*, Vol. 18, 1914, pp. 445-455.)

65. The Bacteriological Standardization of Disinfectants. By H. C. Hamilton and Tatsuzo Ohno. (*American Journal of Public Health*, Vol. 4, No. 6, p. 163.)

66. The Pineal Gland in Relation to Somatic, Sexual and Mental Development. By Carey P. McCord, M.D. (*Journal of the American Medical Association*, July 18, 1914, Vol. 63, pp. 232-235.)

67. The Sero-enzyme Test for Syphilis. By F. W. Baeslack, M.D., M.A. (*Journal of the American Medical Association*, Aug. 15, 1914, Vol. 63, pp. 559-563.)

68. A Case of Contagious Broncho-pneumonia Caused by *Bacillus Coli Communis*. By Edwin M. Stanton. (*American Veterinary Review*, Vol. 14, May, 1914, pp. 233-235.)

69. Local Anesthetics—Some Comparative Physiological Reactions. By Oliver E. Closson. (*Journal of the Michigan State Medical Society*, Vol. 13, Oct., 1914, pp. 587-597.)

POTASSIUM TELLURITE AS AN INDICATOR OF MICROBIAL LIFE.

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Read before the Laboratory Section, American Public Health Association, Colorado Springs, September, 1913.

INTRODUCTION.

The fact that selenium and tellurium compounds, when brought into the living organism, are decomposed by the metabolic processes of the cells with the formation of substances of characteristic odor has been known for a long time. Gmelin (1) and Hansen (2) were the first to investigate this, and toward the close of the nineteenth century the phenomenon had been closely studied and its nature determined upon by Hoffmeister (4) as a synthetical, methylating process. Gmelin, himself, in the course of his investigations, calls attention to the separation of free selenium and tellurium in organisms that had been treated with these metalloids. Beyer (5) investigated this fact more closely, and proved that preparations of selenium and tellurium, in contact with living cells, are decomposed, with a consequent pigmentation of the cells, by absorbing the element.

In the meanwhile, Chabrie and Lapique (3) showed that the microbic cell exhibited a similar relationship, their work, however, being based on mixed cultures. The most significant work was done by Sheurlen (6) and Klett (7), who closely studied the phenomenon with single, microbic individuals, *i.e.*, with pure cultures. From experiments on twenty-seven different kinds of bacteria, they conclude that sodium selenite and sodium tellurite are reduced to metallic selenium and tellurium, respectively, by growing bacteria; and are especially suitable for demonstrating the reducing properties of bacteria. Although there are differences in the extent of reduction among the various kinds of bacteria, in general, all species have the reducing power, and the intensity of reduction is, as a whole, proportional to the intensity

of growth. The reducing action of the bacteria on the selenium and tellurium compounds is produced by the bacterial cell itself (protoplasm) and not by the products of its metabolism.

The work of Scheurlen, Klett and others pointed to the fact that the selenite and tellurite are mostly adapted to prove the reductive power of bacteria. They failed, however, to take into consideration that this reduction of selenium and tellurium compounds can very well serve as an indicator of bacterial life. In fact, their work would lead to the assumption that this reduction is specific to bacteria, which is not the case, for, in our work, we have found that the salts in question can be easily reduced without calling bacteria into play, as through the agency of continued heat, or strongly reducing chemicals.

More recently, Gosio (8) and Belfanti (9) have made an exhaustive study of this phenomenon, particularly with potassium tellurite. While the latter confined his attention almost exclusively to the tubercle bacillus, Gosio investigated various microorganisms and the action of such media as sera, broths, milk, etc. He calls particular attention to the value of the reaction as an indicator of germ life and finds that it is most easily produced in nutrient broth and milk, while in other media in which certain albuminoids predominate, as, for example, sera, the phenomenon is definitely retarded. The addition of a small amount of cane sugar, varying between 0.5 grm. and 1 grm. in 100, he also found to greatly increase the sensitiveness of the "telluric reaction."

EXPERIMENTAL WORK.

A. The Action of Potassium Tellurite on Various Microorganisms.

In our work with potassium tellurite, solutions of the salt in water were employed of such strengths that when 1 cc. was mixed with 10 cc. of culture medium, dilutions were obtained ranging from one part of the tellurite in 2500 parts of water to one part in 300,000 parts of water. These were most conveniently prepared by taking a "stock" solution of one gram of potassium tellurite in 100 cc. of water, filtering slowly through

several thicknesses of filter paper to obtain a clear filtrate, and then diluting the latter as desired. Both the commercial and Merck's highest purity potassium tellurite were used. The latter product, which is a pure, white powder, is readily soluble in water in 1-per-cent solution, and was found to be much more stable and to give uniformly better results with the various organisms.

At first, distilled water was used for the dilutions, being measured with a burette, and the solutions thus obtained were then sterilized by heating in streaming steam for an hour. However, with concentrations of the salt more than one part in 4000 parts of water, a decomposition took place, as shown by the liquid becoming milky and the appearance of fine scaly crystals; on standing, the reagents rapidly darkened with the appearance of black flocculi. The procedure finally adopted was to take the clear "stock" solution, which is itself a germicide, and work aseptically, using sterile pipettes, dilution water, and sterile vessels. Both plain bouillon (containing 2 per cent peptone) and 1 per cent saccharose bouillon, as recommended by Gosio (8), were used as media, except with the tetanus bacillus, where dextrose bouillon in oil was used. Heavy inoculation was made in each case from a young bouillon culture, or an aqueous suspension of the test organism grown on suitable, solid media. Wherever feasible, a number of strains of the organism was employed in the inoculation to avoid individual idiosyncrasies. The manner of recording results is best seen from the accompanying table with *B. coli communis*. In order to form an estimate of the most efficient strength of the potassium tellurite, the reactions obtained have been designated by the numerals 3, 2, 1, 0. In explanation, 3 signifies a strong reaction, as shown by a heavy black precipitate or flocculi; 2, a moderate reaction; 1, a faint reaction, while 0 stands for a negative result, as shown by absence of growth or coloration. Results are in triplicate, and have been recorded after a week's incubation at the optimum temperature for growth. With the exception of *B. coli communis*, the organisms studied are arranged according to the classification of Migula.

1. The Effect of Potassium Tellurite on Cultures of *B. coli communis*.

Exp't'l. numbers.	Dilution.	Medium.	Reaction.
1, 2, 3	1:2500	P.B.*	0, 0, 0.
4, 5, 6	"	S.B.	0, 0, 0
7, 8, 9	1:5000	P.B.	1, 2, 1
10, 11, 12	"	S.B.	0, 0, 0.
13, 14, 15	1:10000	P.B.	2, 2, 2.
16, 17, 18	"	S.B.	1, 1, 2.
19, 20, 21	1:20000	P.B.	2, 2, 3.
22, 23, 24	"	S.B.	2, 2, 1.
25, 26, 27	1:30000	P.B.	3, 3, 3.
28, 29, 30	"	S.B.	3, 3, 3.
31, 32, 33	1:40,000	P.B.	3, 3, 3.
34, 35, 36	"	S.B.	3, 2, 2.
37, 38, 39	1:50,000	P.B.	2, 2, 2.
40, 41, 42	"	S.B.	2, 2, 2.
43, 44, 45	1:100,000	P.B.	2, 1, 1.
46, 47, 48	"	S.B.	2, 2, 2.
49, 50, 51	1:300,000	P.B.	1, 1, 1.
52, 53, 54	"	S.B.	1, 1, 1.
55, 56, 57	Controls	P.B.	0, 0, 0.
58, 59, 60	"	S.B.	0, 0, 0.

*P. B.—Plain bouillon.

S. B.—Saccharose bouillon.

As may be noted from the accompanying table, there is considerable latitude in the strength of tellurite giving the reaction. With *B. coli communis*, the typical reaction seems to be the formation of a heavy black precipitate, sometimes leaving the supernatant liquid clear, but of dark color, more often, however, diffused through the tube of the medium. (Cf. Fig. 1, #1.) A concentration of 1:30,000 of the salt seemed to give the optimum reaction, being apparent even after twenty-four hours' incubation at 37° C.

Microscopical examinations of the colon bacilli thus treated, in hanging drop preparations, showed that the reaction produces distinct morphological changes in the bacillus. The organisms appear very much distended, some being three to four times the normal length. Instead of being straight, many are curved, resembling spirilli. The most striking modification, however, is the presence of black granules, resembling those of *Bact. diphtherie* when stained by Neisser's stain, but having from three

to four granules present in every organism. Especially in older cultures showing the reaction, the bacteria seemed grouped to-

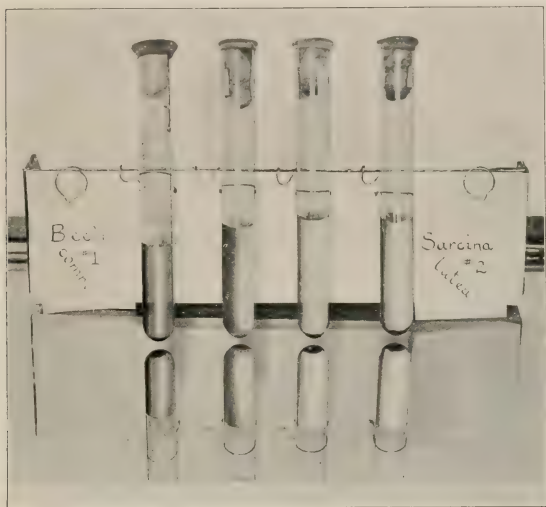


FIG. 1.

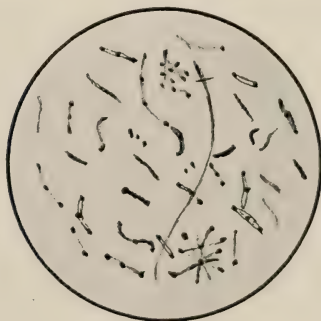


FIG. 2.

B. coli communis.

Treated with Potassium Tellurite.
Drawing from Hanging Drop Preparation.

gether as if they were agglutinated. Saccharose bouillon, as a whole, did not give any better reaction than did the plain bouillon.

2. *Streptococcus pyogenes*.

In working with the streptococcus, a "stock" vaccine was used, containing eleven different strains of streptococci. Here again, the action was rather slow in appearing and seemed best at 1:50,000. It is shown macroscopically by a finely granular, black precipitate clinging tenaciously to the bottom of the tube, while the supernatant bouillon is usually clear and not darkened.

Examined in the "hanging drop" there is a preponderance of short chain forms, as compared with long chains in the control tubes containing no tellurite. These short chains are composed mostly of three or four black beads, with perhaps several uncolored beads where the reaction has not been completed, all of which are the normal size.

3. *Micrococcus pyogenes albus*.

4. *Micrococcus pyogenes aureus*.

5. *Micrococcus pyogenes citreus*.

For convenience, the above three races of staphylococci were studied together, with the view, if possible, of noting any distinctions apart from their chromogenic power. In a general way, the reaction given by both the *aureus* and *albus* varieties were quite similar, that given by the former being the more pronounced. Both, however, were rather weak. The optimum concentration seems to be about 1:100,000, although good results are obtained at 1:50,000.

Seemingly in marked contrast, a strong reaction was obtained with the *Micrococcus pyogenes citreus* in every dilution studied. It would appear that, in addition to chromogenesis, this behavior towards potassium tellurite possibly might be utilized as a means of differentiation between the varieties of the pyogenic staphylococci.

In routine procedures involving laboratory diagnosis, as in the preparation of autogenous vaccines and other manipulations, it is necessary to identify the *Micrococcus pyogenes* group with the least possible delay. Where there is relatively slow pigment formation, the use of potassium tellurite might be found to possess some value.

Macroscopically, the reaction with the staphylococci shows a

similarity of appearance to that obtained with the *Streptococcus pyogenes*, the supernatant bouillon being clear, with a thick, viscous, black sediment. Examined in the "hanging drop," the "tellurited" organisms appear somewhat smaller than the normal,

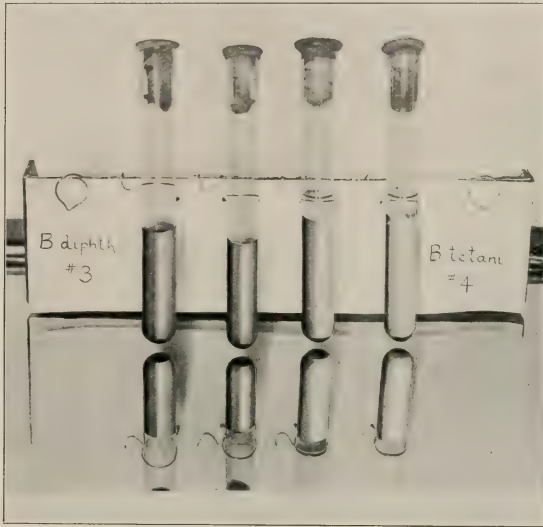


FIG. 3.

control cultures, and are shown usually by three or four black cells, the remaining cocci in the group being unaffected.

6. *Sarcina lutea*.

Sarcina lutea seemed to react well with potassium tellurite, the tubes showing a characteristic granular sediment of a black mottled appearance, with the supernatant liquid clear. (Cf. Fig. 1, #2.) Examined under the microscope in a "hanging drop," the usual cubical packets are seen, having mostly one or two uncolored cells, the rest of the cocci being entirely black. The reaction, as a whole, is rather quick in appearing, being well developed in forty-eight hours, and seems to be best at 1:40,000.

7. *Bacterium diphtheriæ*.

Although the "tellurite reaction" is rather slow in making its appearance with *Bact. Diphtheriae*, it is, nevertheless, very marked. In the higher concentrations, particularly 1:10,000, the appearance of the tube was almost black, with a heavy black precipitate. The most typical reaction seems to be at 1:50,000, and is shown by a dense, black precipitate clinging tenaciously to the bottom of the tube, while the supernatant medium is cloudy, and frequently colored brown. (Cf. Fig. 3, \pm 3.) Apparently no anti-septic action was exerted even at 1:2500, while 1:300,000 also gave a good result, indicating a wide latitude in which the "tellurization" manifests itself. This relatively great tolerance of potassium tellurite by the diphtheria organism has more recently



FIG. 4.

Bact. diphtheriae.

Treated with Potassium Tellurite.
Drawing from Hanging Drop Preparation

found practical application in the differential diagnosis of diphtheria by means of the Conradi-Troch "Tellurium plate." (Cf. Wagner (10), Schürmann and Hajós (11).)

Examined in the "hanging drop," the organism studied, which was of the Westbrook "A" type, appeared as if stained by the Neisser method. The granules were especially apparent, as were also the "V" shaped or "snapping forms," and frequent, extra large, individual cells, similar to those obtained with five-hour cultures on blood serum at 37° C. On the whole, the organisms appeared much larger than when examined in a normal, stained preparation, and also larger than those of the "control cultures."

8. *Bacterium tuberculosis* (Homo.).

In the work with this organism, the bacteria were first grown on glycerine agar, then accustomed to plain and saccharose bouillon by several transplantations. Owing to the scanty growth of the organism in the culture media employed, as shown by the "control tubes," the reaction in no case was very strong. Macroscopically, it appeared as an almost metallic, black, flaky deposit, or black ring, at the bottom of the bouillon tubes, the remainder of the medium being clear.

On microscopical examination, the "treated" tubercle bacilli showed several black granules, sometimes appearing as bipolar. Occasional branched forms, such as are encountered in the sputum of those suffering from pulmonary tuberculosis, are also found in some of the fields. The action is very slow in making its appearance, and is not fully developed before a week's time. Here, also, a wide range in the dilutions giving the results is found, the best being at about 1:10,000.

9. *Bacillus subtilis*.

As would be expected, the action is slower with *B. subtilis* than with *B. coli communis*, the first evidences of blackening appearing only after forty-eight hours. The dilution giving the best results seems to be about 1:50,000, although the differences with the various concentrations are not as marked here as with *B. coli communis*.

Macroscopically, the action with *B. subtilis* appears as small, black clumps at the bottom of the broth tube with the supernatant liquid entirely clear, and usually a blackened, wrinkled pellicle at the surface of the medium. Examined microscopically in a "hanging drop" preparation, both individual bacteria and zooglea films can be seen. In both cases, the organisms are considerably smaller than the untreated forms, and are colored a uniform black.

10. *Bacillus typhosus*.

Contrary to what would be expected, the results obtained with the typhoid bacillus differed materially among various strains, and especially from *B. coli communis*. On this account, we are making a special study of the "tellurite reaction" with the colon-typhoid group, the results of which we hope to report upon in the near future.

The typhoid bacillus seems to be peculiarly sensitive to the antiseptic action of the tellurite. Below 1:50,000 there is very little reaction, while the best results are obtained at 1:100,000. The appearance of the typical reaction is very similar to that of the colon bacillus—a powdery, black precipitate at the bottom of the tube, usually diffused through the supernatant liquid, giving it a brownish-gray tinge. On microscopical examination, however, a marked difference is apparent. The “tellurited” organisms appear about two-thirds as large as the normal typhoid bacilli, are non-motile, and instead of the four granules of the colon bacillus, these have a single, round, black granule at each pole. Occasionally an organism is seen having three such granules, while others are found considerably smaller in size and entirely black in color. The reaction is comparatively slow in appearing.

11. *Bacillus abortus* (Wien I).

A culture of *B. abortus* being available at the time of this investigation, it was thought advisable, in the interests of completeness, to compare the action of this organism to potassium tellurite. As was expected, the reaction with *B. abortus* was not very pronounced, owing in a great measure to the fact that the organism, itself, requires special, anaërobic cultivation. The results obtained, however, seem to justify the conclusion, that the bacillus of contagious abortion gives the “tellurite” reaction. About 1:50,000 seemed to give the best results. At this concentration it showed as a slight, brownish turbidity with a blackened sediment in the tubes. Microscopically, the bacilli, which normally are well isolated, appear grouped together in twos and threes in a row, so that at first glance they might be mistaken for darkened bacilli with several spores, or light-colored granules.

12. *Bacillus prodigiosus*.

Bacillus pyocyaneus.

B. pyocyaneus and *B. prodigiosus*, in mixed culture, gave a marked reaction with tellurite, particularly in the concentrations ranging from 1:5000 to 1:20,000. Macroscopically, this was shown by a decided blackening, usually diffused throughout the entire medium with a black ring at the surface, but appearing as a black sediment in the higher dilutions. Chromogenesis is not affected, as is evidenced by the fact that the tubes showing the

black deposit still had the greenish fluorescence and the red ring characteristic of *B. pyocyaneus* and *B. prodigiosus*, respectively, when grown in bouillon. Examined microscopically in the hanging drop, the smaller, oval *B. prodigiosus* is found to be uniformly blackened and only slightly motile. The larger *B. pyocyaneus* appears with two or more black granules, the motility being unaffected. The reduction is very rapid, being plainly evident after twenty-four hours' incubation.

13. *Bacillus mesentericus*.

For *B. mesentericus*, a concentration of about 1:30,000 seems to give the best reaction with potassium tellurite, although a good result is obtained even at 1:300,000. Concentrations stronger than 1:10,000 have an apparent, antiseptic action. The action is rather slow in appearing, and is shown by a dark, gray-black sediment, the supernatant medium being clear.



FIG. 5.
B. tetani.
Treated with Potassium Tellurite.
Drawing from Hanging Drop Preparation.

Microscopically, the bacilli appear much smaller than normal with bipolar, black granules. Occasionally, an organism is found uniformly colored black. The motility is less than normal, and spore formation is not so apparent.

14. *Bacillus tetani*.

Owing to the strict, anaërobic requirements of the tetanus bacillus, the ordinary technique could not be observed. Accordingly, glucose bouillon with neutral oil was chosen as the medium,

and inoculated with four drops of a good growing bouillon culture of the organism. This was then incubated for two days at 37° C., and finally potassium tellurite added, as usual, to give the various concentrations.

Unlike most of the other organisms studied, the best reaction of the tetanus bacillus was at the strongest concentration employed—1:2500. As would be expected, the reaction is slow in appearing, and is first shown by a brown ring near the top of the culture tube. This gradually extends down to the bottom, giving a black flaky deposit, with the supernatant medium brown and turbid. (Cf. Fig. 3, #4.)

Examined in "hanging drop" preparation, almost the first thing noticed is the decided, granular appearance of the bacillus. Exceptionally long forms, showing as many as twelve black granules, are frequent, and, in some cases, show the characteristic "drum-stick" spore. The large spores, sometimes noted with this organism, are also seen, seemingly with a black spot in the center of the spore. The reaction takes at least a week before completion, but is distinct even at 1:100,000.

15. *Spirillum tyrogenum*.

The reaction with this organism was very slow in appearing and not very marked. The tubes showing the most typical results had either small, black granules in the sediment at the bottom of the tube, or a black ring. On microscopical examination, the spirilla were found to be smaller than normal, sluggishly motile, and colored uniformly black. About 1:40,000 seems to be the concentration giving the best results.

16. *Saccharomyces cerevisiæ*.

Saccharomyces glutinis.

Saccharomyces albus.

For studying the action on yeasts, a culture was used, consisting of the ordinary beer yeast *Saccharomyces cerevisiæ* together with two so-called "wild yeasts" *Saccharomyces albus* and *Saccharomyces glutinis*. The material used for inoculation was obtained by washing off a twenty-four hour, 20° C. agar slant culture into sterile water.

The "tellurite" action with yeasts is very rapid, being distinctly visible after incubating for eighteen hours at room temperature, which is the most suitable for growing the yeasts. With the

exception of the highest dilution (1:300,000), all of the tubes showed a characteristic, heavy, black deposit, frequently extending up through the culture medium, with a black ring at the

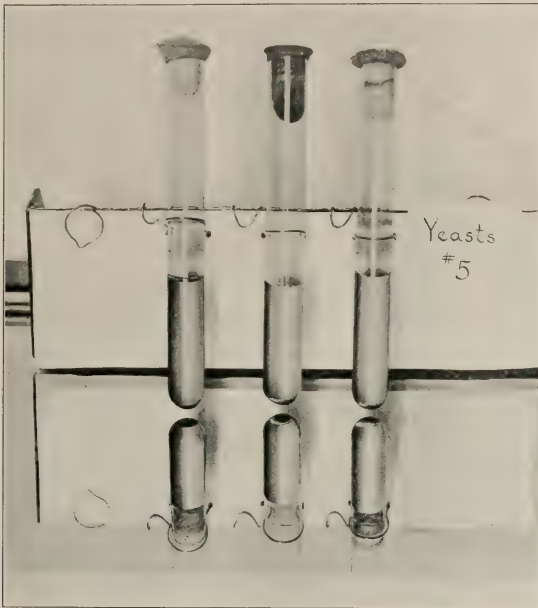


FIG. 6.

surface. (Cf. Fig. 6.) The cells of the *albus* and *glutinis* were, in most instances, found to be stained deeply black, while that of the *cerevisia* showed black granules, the remainder of the cells being unaffected. No alteration in size or tendency towards involution forms was noticed.

17. *Penicillium glaucum*.

Aspergillus.

For the study of the action on molds, the bouillon tubes were inoculated with the ordinary blue-green mold—*Penicillium glaucum*, together with a variety of *Aspergillus*, species unknown.

THE ACTION OF POTASSIUM TELLURITE ON VARIOUS MICROORGANISMS

Organism.	Macroscopical appearance.	Microscopical appearance.	Concentrations giving reaction and optimum concentration.
1. <i>B. coli communis</i> .	Heavy black ppt., diffused through medium. Action rapid and strong.	Bacilli larger, curved and clumped. 3-4 black granules.	1:10,000—1:300,000 1:300,000
2. <i>Strep. pyogenes</i> .	Finely granular black ppt. supernatant bouillon clear. Action slow and moderate.	Normal size, short chains. 3-4 black beads in chain.	1:40,000—1:300,000 1:50,000
3. <i>Mic. pyog. albus</i> .	Small, viscous, black sediment; supernatant bouillon clear. Action slow and feeble.	Smaller size with 3-4 black cells.	1:40,000—1:300,000 1:100,000
4. <i>Mic. pyog. aureus</i> .	Small, viscous, black sediment; supernatant bouillon clear. Action slow and feeble.	Smaller size with 3-4 black cells.	1:40,000—1:300,000 1:100,000
5. <i>Mic. pyog. citreus</i> .	Thick, viscous, black sediment; supernatant bouillon usually clear. Action strong.	Smaller size with 3-4 black cells.	1:2,500—1:300,000 1:50,000
6. <i>Sarcina lutea</i> .	Characteristic, gran. black sediment. Supernatant liquid clear. Action rapid and strong.	Normal size with blackened cells.	1:2,500—1:300,000 1:40,000
7. <i>Bact. diphtheria</i> .	Dense, clinging, black ppt. Supernatant liquid cloudy and brown. Action slow but strong.	Bacteria larger with numerous black granules.	1:2,500—1:300,000 1:10,000
8. <i>Bact. tuberculosis (Homo)</i>	Metallic, black, flaky deposit. Supernatant liquid clear. Action slow and feeble.	Bacteria branched and granulated.	1:15,000—1:300,000 1:10,000
9. <i>B. subtilis</i> .	Small, black clumps; supernatant liquid clear with black pellicle. Action rapid and strong.	Bacilli smaller and uniformly black.	1:20,000—1:300,000 1:50,000

THE ACTION OF POTASSIUM TELLURITE ON VARIOUS MICROÖRGANISMS
Concluded.

Organism.	Macroscopical appearance.	Microscopical appearance.	Concentrations giving reaction and optimum concentration.
10. <i>B. typhosus</i> .	Powdery, black ppt. diffused through medium. Action slow and moderate.	Smaller size with one black polar granule.	1:50,000—1:300,000 1:100,000
11. <i>B. abortus</i> .	Slight brownish turbidity with black sediment. Action slow and feeble.	Bacilli grouped and entirely blackened.	1:5,000—1:300,000 1:50,000
12. <i>B. prodigiosus</i> . <i>B. pyocyaneus</i> .	Black sediment, diffused through medium with black ring. Action rapid and heavy.	Normal size; <i>B. prod.</i> blackened and <i>B. pyocy.</i> granulated.	1:5,000—1:300,000 1:50,000
13. <i>B. mesentericus</i> .	Dark, grey-black sediment; supernatant liquid clear. Action slow and moderate.	Bacilli smaller with bipolar, black granules.	1:20,000—1:300,000 1:30,000
14. <i>B. tetani</i> .	Black, flaky deposit; supernatant liquid brown and turbid. Action very slow but heavy.	Bacilli larger with numerous black granules.	1:2,500—1:100,000 1:2,500
15. <i>Spir. tyrogenum</i> .	Small, black granules or black ring. Liquid above, clear. Action slow and feeble.	Org'ns. smaller and blackened. Motility affected.	1:2,500—1:300,000 1:40,000
16. <i>Sacch. cerevisiæ</i> . <i>Sacch. albus</i> . <i>Sacch. glutinis</i> .	Characteristic, heavy, black sediment; supernatant liquid blackened and with ring. Action very rapid and strong.	Normal size, <i>Sacch. alb.</i> and <i>Sacch. glut.</i> blackened, while <i>Sacch. cerev.</i> granulated.	1:2,500—1:300,000 1:50,000
17. <i>Penicillium glaucum</i> . <i>Aspergillus</i> .	Heavy, black, felted mass, extending into medium. Action very rapid and strong.		1:2,500—1:300,000 1:50,000

Average optimum concentration, 1:50,000.

The incubation here, also, was at room temperature, but the reactions obtained were much stronger than with the yeasts. In almost every instance, the surface of the tube was covered with a heavy, black, felted mass, frequently extending down into the medium itself. Apparently the various concentrations had very little influence on the intensity of the reaction, as it was as marked at 1:2500 as at 1:300,000, and in each case appeared in twenty-four hours.

B. *General Considerations of the "Tellurite Reaction."*

As true of any "vital reaction" where chemical changes are produced through the agency of living material, both physical and chemical factors have a marked influence on the action of potassium tellurite on microorganisms. Essentially, this is a reduction from the tellurite to metallic tellurium, probably with the formation of hydrogen and hydrogenated compounds. This is evidenced by the fact that with the *Bact. tuberculosis*, not only did we find the black metallic precipitate indicative of the tellurium, but several of the tubes had a garlic-like odor analogous to the arsines, and which in all probability were hydrogenated tellurium compounds or "tellurines." Theoretically, then, the presence of saccharose in the medium as recommended by Gosio (8) should make it more easy for the bacteria to reduce the tellurium salt through the formation of special fermentation products. Our work, however, has failed to find any material advantages resulting from the use of saccharose bouillon. In fact, with *B. mesentericus*, *B. abortus*, *Streptococcus pyogenes* and *B. coli communis* the plain bouillon seemed even slightly better than the saccharose.

The presence of acids or alkalies, as would be supposed, interferes with the "tellurite reaction," the acids more so than the alkalies. This is due not only to actual inhibitory action upon the bacteria, but also to a chemical decomposition of the tellurite, as shown by the formation of a white precipitate. Similarly, strong heat, cold, and light have both an action on the salt and an action on the bacteria. With solid media, such as agar, addition of potassium tellurite 1:5000 to melted agar at 60° C. gave a decided brown color after solidification, while no effect was noticed when the agar was kept at 30° C.

It is also apparent that to obtain good results with potassium

tellurite, the organism must be in an active state of metabolism. Any factor influencing the growth of the microbes will consequently influence the "tellurium reaction." The presence of dilute antiseptics, by retarding the growth of the bacteria, hinder the reduction phenomenon. In this connection, it must be borne in mind that the potassium tellurite, itself, has a relatively strong germicidal action. A germicidal assay of this salt made by our colleagues, H. C. Hamilton and T. Ohno, by the Hygienic Laboratory Method, gave a phenol coefficient of 6. For comparative purposes, an "antiseptic value" test was made at the same time by inoculating 5 cc. of the tellurite in various dilutions with 0.1 cc. of a twenty-four-hour culture of *B. typhosus* (Hopkins) in bouillon. After forty-eight hours' contact, a subculture was planted in bouillon, and its condition recorded after forty-eight hours' time for growth in the medium. The results indicated that potassium tellurite can be used as an antiseptic in a concentration of 1:2500, provided the organisms are exposed to the action of the solution for at least forty-eight hours.

It is evident from our results that the capacity for reducing potassium tellurite varies with the different microorganisms. Some, as, for example, the yeasts, molds, and *Bact. diphtheria*, are especially energetic, while others, as, for example, *B. abortus*, and *Spirillum tyrogenum*, gave a weaker action. Including our work with the colon-typhoid group, we have studied over twenty-five species of microorganisms. In every instance, the capacity for decomposing the potassium tellurite was present in a manner easily perceivable by the naked eye. Naturally, where bacteria are present in the spore form, no reduction of the tellurite can take place. It seems fair to assume, then, that the power of reducing potassium tellurite with the formation of characteristic black compounds is common to all microorganisms in an active state of metabolism.

The best results seem to be obtained with those organisms commonly found as sources of contamination, as the colon bacillus, the hay bacillus, yeasts, molds, and others. Therefore, potassium tellurite appears to be well adapted as an indicator of microbial life in general, but is especially suitable as an indicator of ordinary bacterial contamination occurring under practical conditions.

A general survey of the reaction with the various microorganisms studied seemed to show that an average concentration of 1:50,000 is the most suitable for the use of potassium tellurite as an indicator. Using such a concentration of the salt, we have tested for sterility numerous aseptic biological and chemical preparations, including sera, bacterial vaccines, alkaloidal and drug products under varying conditions. The criterion in each case was the presence or absence of a black precipitate or coloration. Controls were run at the same time, and the results checked in each instance by growing the material both aërobically and anaërobically in suitable culture media. The material was so selected or treated that about one-half was actually contaminated. In practically every case, the cultural results and those obtained by the indicator were in entire agreement.

The possible application of potassium tellurite in hypodermatic products, brought up the question of its toxicity and irritating action. In experiments which were conducted by our colleague, O. E. Closson, with guinea-pigs, 0.3 cc. of 1-100 solution produced marked congestion at the injection site when examined four days after. One cc. of 1-2000 showed locally a congestion at the end of forty-eight hours; 2 cc. of 1-5000 showed locally only a very slight inflammation at the end of forty-eight hours; 6.5 cc. of 1-15,000 showed locally no signs of the injection at the end of forty-eight hours. Contrary to the findings of Gosio (8), the minimum lethal dose for guinea-pigs at the dilution 1-2000 was 0.003 gm. per kg. Autopsy on the animals killed with the salt showed a gastro-intestinal inflammation; the site of the injection was inflamed, and had a dark deposit with a garlic-like odor.

A dog receiving subcutaneously 5 cc. portions of 1-1000, 1-2000, 1-1000, 1-8000, 1-15,000, and 1-30,000, examined at the end of forty-eight hours, showed some inflammation at the point of injecting the 1-1000 solution, just a trace at the 1-2000, and no indication at the points where the more dilute solutions were injected. A dog receiving 2 cc. portions of the same solutions, examined four days later, showed a very considerable congestion at the site of injecting the 1-1000 solution, just a trace at the 1-2000, and at the 1-8000, and absolutely none at the other points of injection.

CONCLUSIONS.

1. Nearly all of the more common microorganisms react with potassium tellurite, forming characteristic, black compounds.
2. This capacity depends on an active state of metabolism of the reacting organism, and the action is, in all probability, a reduction of the tellurite.
3. The "tellurite reaction" can be used as an indicator of microbial life, and is especially suitable for revealing microbic contamination.
4. A dilution of 1:50,000 of the salt seems to be most suitable for its action as a general microbic indicator. In this concentration it produces no irritative action when introduced into test animals.

BIBLIOGRAPHY.

1. Chr. Gmelin—"Versuche über die Wirkungen des Baryts. Strontians, u.s.w." 1824, Tübingen (from Ztschr. f. Hyg. u. infkr., 1905, V. 51, p. 72).
2. K. Hansen—"Versuche über die Wirkung des Tellurs auf den lebenden Organismus." *Annalen der Chemie und Pharmacie*, 1853, V. 86.
3. C. Chabrie und L. Lapique—"Sur l'action physiologique de l'acide salicieux." *Compt. rend. de l'Acad. des Sciences*, 1890, V. 110.
4. Hofmeister—"Ueber Methylierung in Thierkörper," *Archiv. f. experim. Pathol. u. Pharmacol.*, 1894, V. 33.
5. I. L. Beyer—"Durch welchen Bestandtheil der lebendigen Zellen wird die Tellursäure reducirt?" *Archiv. für Physiologie von Dubois, Reymond*, 1895.
6. Scheurlen—"Die Verwendung der selenigen, und tellurigen Säure in der Bakteriologie," *Ztsch. f. Hyg. und Infkr.*, 1900, V. 33, p. 135.
7. A. Klett—"Zur Kenntniss der reducirenden Eigenschaften der Bakterien," *Ztschr. f. Hyg. und Infkr.*, 1900, V. 33, p. 137.
8. B. Gosio—"Indicatoren des Bakterien Lebens u. ihre prakt. Bedeutung." *Ztschr. f. Hyg. und Infkr.*, 1905, V. 51, p. 72.
9. S. Belfanti—"Die vitale Reaktion nach Gosio beim Tuberkelbazillus." *Ztschr. f. Chemotherapie und verwandte Gebiete*, 1912, V. 1, pt. 2, p. 113.
10. G. Wagner—"Münchener medizinische Wochenschrift, 1913, V. 60, No. 9, p. 457.
11. W. Schürmann und E. Hajos—"Deutsche medizinische Wochenschrift, 1913, V. 39, No. 17, p. 786.

FURTHER STUDIES WITH REFERENCE TO SPIROCHETES OBSERVED IN SWINE.

STUDIES ON HOG CHOLERA.*

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With 2 figures and 5 curves in the text.

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INTRODUCTION.

Spirochata suis has been described in former reports as an organism measuring approximately 6 to 10 microns in length and about 0.3 to 0.6 microns in width. It is a typical spirochete, consisting of 5 to 10 convolutions, and the spirals are fixed. In cultures, some individual organisms may show only 3 or 4 convolutions. In fresh preparation, when studied by the dark field, the motility is often very active and characteristic. While the organisms tend to move backward and forward in the same plane, they often exhibit a rapid, awkward appearing movement of the fixed spirals, not unlike the motion of hinge joints.

The above brief description applies to this organism as it is found in local external lesions or intestinal mucosa of swine suffering from hog cholera (1). A spirochete-like form which has been reported by us (2, 3), and confirmed by Arnheim (4), as being found by the dark field method in the blood of cholera hogs, differs in morphology. It is relatively much thicker and its motility is undulating, although the spirals are fixed. As suggested in our former reports (*Journ. Infec. Diseases*, Vol. 12, 1913, p. 212, and Vol. 13, p. 466): "Whether or not some of these spirochetes in the intestinal lesions bear any relation to those present in the blood of cholera hogs is, of course, an open question. It is possible that the spirochetes found in the blood enter the lymph and blood stream at an early stage of the ulcer formation. This would offer a possible explanation of their comparatively small number as seen on any one blood mount." "Results lead us to believe that the spirochete observed . . . in the blood of cholera hogs is a transitional form."

It occurs in very few numbers, as shown by careful dark field study of hog cholera blood, and, up to the present time, attempts to cultivate it from the blood as well as to secure satisfactory stained preparations, have been unsuccessful. It should be stated,

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however, that very little attention has been given to the study of the blood of cholera hogs during the past year, observations having been confined almost exclusively to spirochetes as they appear in external or intestinal lesions from cases of hog cholera.

From two different sources, "*Spirochaeta suis*" has been suggested as a name for spirochetes observed in swine. Bosanquet (5), in his book, refers to the observations of Dodd (6) and Cleland (7), and Neveau-Lemaire (8), in a compilation, also mentions the reports of both of the above investigators.

Bosanquet's reference is as follows:

"Spirochaetes in the Pig. (*Spirochaeta suis*?)

"Dodd found spirochaetes in a disease affecting the skin of the pig, communicated by contact and inoculable in the skin of a healthy animal. The organisms (Fig. 72) were not found in the blood; they disappeared and reappeared in the cutaneous lesions from time to time.

"Cleland observed spirochaetes in tumours occurring at the site of castration in pigs. The organisms were from 6 to 12 μ in length and exhibited three or four irregular curls. Along with these organisms were large bacillary forms, some of which were slightly undulating and suggestive of being modified forms of the spirochaetes."

In the book of Neveau-Lemaire, reference is made to spirochetes in swine as follows:

"*Spirochaeta suis*—We provisionally give this name to a spirochete described by Burton Cleland (Note on spirochaetes in castration tumours of pigs. Parasitology. A supplement to the *Journ. of Hygiene*, Vol. 1, No. 3, p. 218-219), in tumours following castration in the pig. This organism measures from 6 μ , and even less, to 12 μ in length; its width scarcely exceeds that of a Koch bacillus; it presents three or four irregular spirals. It is found in the yellowish-brown pus which drains from the tumors in question, the size of which may vary from that of a hen's egg to that of a tennis ball.

"These phenomena following castration are relatively frequent in the pigs of western Australia. However, it is still impossible to say whether the spirochete plays a role in the pathogenesis of these tumors or whether it is simply an ordinary saprophyte introduced into the wound at the time of castration.

"Sydney Dodd (A Disease of the Pig, Due to a Spirochete. *Journ. of Comparative Pathology*, Vol. 19, 1906, p. 216) has described an ulcerative condition of the skin of the pig, in which he has observed spirochetes very similar to, and perhaps identical with, that which concerns us (Mathis and Leger have described at Le Touquin a spirochete of the rabbit; *Spirochaeta Kullbetti*. Soc. de Biol., 11 Fevr. 1911)."

We believe the species which we have observed in hogs

affected from cholera is not the same as those described by Dodd and Cleland. Dodd and Cleland both described the form which they observed as consisting of 3 or 4 irregular convolutions. Therefore, in order that the rules of nomenclature shall be properly observed, it is necessary that some other name than *Spirochæta suis* be used in designating the organism with which we are working. Hereafter we shall use the name *Spirochæta hyos* (from the Greek, meaning spirochete of the pig).

In conjunction with the later studies of this spirochete in the blood, many observations have been made by means of the dark field of material taken from the intestinal ulcers of hogs dead from hog cholera during the course of our experiments for several months. Scrapings from the intestinal mucosa and contents of the crypts in the ceca of normal hogs, some of these being susceptible to hog cholera, others immune to the disease, have also been examined at every opportunity. As a result of this work, we have arrived at the following tentative conclusions:

1. The mucosa of the large intestine, particularly that of the cecum, of normal hogs includes in its flora non-pathogenic spirilla and spirochetes, relatively large forms predominating.

2. In the ulcerated patches of cecal mucosa and in the crypts, near the ileo-cecal valve, of hogs dead from cholera is localized a constant species of spirochete, which is pathogenic for swine and which plays an important part in the production of hog cholera.

3. The crypts and healed ulcers of hogs actively immunized against hog cholera may contain, for a variable period of time after immunization, in addition to non-pathogenic spirochetes, the same species of pathogenic spirochete referred to above.

Positive dark field findings of spirochetes in intestinal ulcers and mucosa of cholera hogs are in accordance with the results reported by von Rütther (9), Uhlenhuth and Haendel (10), Betegh (11) and Arnheim (4). We have been unable to confirm the findings of spirochetes in the bile of cholera hogs, as reported by Uhlenhuth and Haendel.

On account of the fact that efforts to secure cultures or satisfactory stained preparations of *Spirochæta hyos* from the blood of cholera hogs were not successful, and because of the large amount of bacterial contamination present in the intestinal

lesions, attempts have been made to find localized foci of the organism elsewhere in the diseased body. Microscopical preparations and cultures were made from the spleen, liver, lymphatic glands, spinal cord, cerebro-spinal fluid, bile and urine. These attempts were unsuccessful except in one instance. In one case (Hog 615, inoculated subcutaneously with exudate from local lesion, Hog 599), the dark field revealed in the inguinal lymph gland a spirochete resembling the assumed pathogenic form. In many of the dark field preparations numerous granules were observed, but it was impossible to draw any definite conclusions as to their origin and relationship to *Spirochæta hyos* or to tissue elements.

Finally, when an opportunity presented itself, dark field examinations were made from the exudate of external lesions on the leg and ear of a case of cholera of the chronic type (Hog 583, May 8, 1913). This material contained spirochetes, presumably of one species, in large numbers. So far as we know, similar observations have never been reported except by Dodd (6) of Pretoria, Transvaal, and Cleland (7) of Australia. In describing "A Disease of the Pig, Due to a Spirochete," Dodd states that he found a spirochete in the cutaneous lesions of a number of pigs. The disease could not be transmitted experimentally by the inoculation of blood from an infected animal, but was transmitted by actual contact. The infection was fatal to several pigs. Autopsy revealed nothing except the local skin lesions and general anemia. The disease appeared to be some cutaneous infection due to the spirochete found in the local lesions. Scrapings from the skin of healthy hogs and from the normal skin of the affected pigs were found to contain no spirochetes. Cleland reported finding a spirochete in tumors following castration of pigs. He stated that the presence of spirochetes in these tumors is relatively frequent in Australia, and that at that time it was impossible to determine whether the spirochetes play a role in the pathogenesis of these tumors or whether they are ordinary saprophytes introduced into the wound at the time of castration.

The purpose of this paper is to present data which served as a basis for the tentative conclusions noted above. The following notes include recorded positive and negative findings resulting from the dark field study of the intestinal mucosa and external local lesions of normal and diseased hogs.

Notes on Positive and Negative Findings, *Spirochaeta Hyos*,
in Tissues of Normal and Diseased Hogs.

POSITIVE FINDINGS.

Various dark field observations have been made of spleen, lymphatic glands, liver, lungs and other tissues, as well as bile

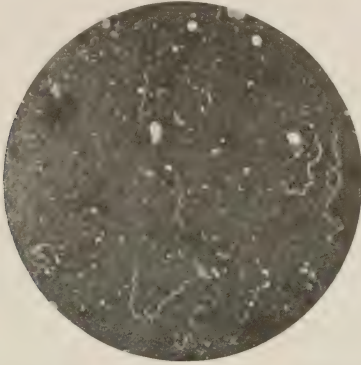


Fig. 1. Photomicrograph of *Spirochaeta hyos* in exudate, ear, lesion, hog 665, X 1200. India ink preparation (Burri method).

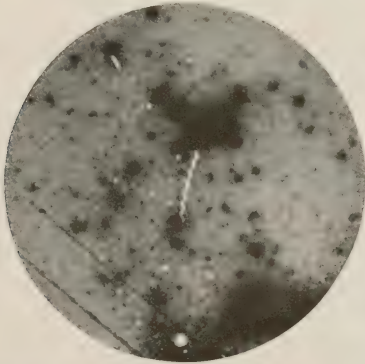


Fig. 2. Photomicrograph of *Spirochaeta hyos* in ulcers from cecum, hog 728, X 1200. India ink preparation (Burri method).

and urine of cholera hogs. Some of the tissues have also been sectioned and stained. In so far as our studies have progressed, the presence of *Spirochaeta hyos* has been demonstrated with uniformity only in the intestinal mucosa and external local lesions of cholera hogs. From the intestinal lesions cultures of *Spirochaeta hyos* have been grown through three generations on artificial media and typical hog cholera produced from the cultures (Journ. Inf. Dis., Vol. 13, 1913, p. 462).

The presence of *Spirochaeta hyos* in tissues of normal and diseased hogs has been noted in 111 cases as shown in the following table. All observations have been made from fresh tissue or tissue exudate, by means of the dark field.

Table I.
Spirochaeta hyos. Positive findings in tissues of diseased and Normal Hogs.

No. cases	Treatment	Clinical condition	Autopsy findings	Condition of animals	Tissues examined by Dark-Field method	Nature of results, as control observations
87	Exposed to Hog cholera by injection of virus	Typical Hog cholera	Positive	H. cholera	Ulcers and scrapings from mucosa of cecum	Positive
22	idem	Typical H. C. complicated by septicaemia	idem	H. cholera and septicaemia	idem	idem
1	"	Typical Hog cholera	"	H. cholera	Inguinal gland	"
1	"	idem	"	idem	Testes	"
22	"	"	"	"	Local lesion ear. Necrotic tissue around ear tag	"
33	"	"	"	"	Local lesions on legs or abdominal region	"
5	Treated with exp. vaccine. Exposed to H. C., released, killed and autopsied	Mild H. C. and recovery or reaction following exp. vaccination	Normal	Immune	Crypts in cecum	Positive, assuming that these immunes were "carriers"

No comments on the above tabulated results are necessary except perhaps brief detailed data concerning 5 cases of immune

animals, in the intestinal crypts of which *Spirochæta hyos* was found. These animals were treated as follows:

Hog A.

H. S. V.* (IV. 11). Cholera (acute).

Mercury preparation (IV. 16).

Mercury preparation (IV. 24). Recovery.

Released (IV. 30).

Killed and examined (VI. 27). *Sp. hyos* found in crypts in cecum.

Hog 649.

Exp. vaccine (VIII. 25).

Exp. vaccine (IX. 9). Strong reaction.

H. C. S. (IX. 23). Immune.

Killed and examined (XI. 18). *Sp. hyos* present in crypts of cecum.

Hog 633.

Exp. vaccine (VIII. 28). Strong reaction.

H. C. S. (IX. 23). Reaction, immune.

Killed and examined (XI. 16). *Sp. hyos* present in crypts of cecum.

Hog 668.

Exp. vaccine (IX. 30). Mild reaction.

Exp. vaccine (X. 7).

H. C. S. (X. 18). Immune.

Killed and examined (XI. 16). *Sp. hyos* present in crypts of cecum.

Hog 678.

Exp. vaccine (X. 11). Reaction.

Exp. vaccine (X. 23).

Exp. vaccine (XI. 3).

H. C. S. (XI. 13). Reaction, immune.

Killed and examined (XI. 22). *Sp. hyos* present in crypts in cecum.

These immune hogs, on being killed and material from crypts of ceca submitted to dark field examination, gave positive findings after the following intervals subsequent to exposure:

Hog A, after 11 weeks.

" 649, " 8 "

" 633, " 7 " and 5 days.

" 668, " 4 " " 1 "

" 678, " 10 days.

These five animals were normal and in excellent condition when killed and examined. The presence of *Spirochæta hyos* in the crypts of the ceca would possibly indicate that they should

*H. S. V. and H. C. S. occurring in these notes indicate horse serum virus and hog cholera serum, respectively.

be classed as "carriers," if it were assumed that the organism in question is of etiological significance. Some investigators are inclined to recognize the probability of immune hogs acting as carriers of hog cholera virus for a variable period of time following active immunization. There is no serious reason why such a phenomenon should not apply to hog cholera as well as to some other specific infectious diseases. Should such a hypothesis appear unreasonable, it might be suggested that in the case of these five immune animals *Spirochæta hyos* still remained localized in the intestine in an attenuated condition.

A suspension was made of material from the crypts in the cecum of Hog A, which contained forms morphologically similar to *Spirochæta hyos*, 11 weeks after the animal had received hog cholera virus or about 8 weeks after full recovery. 2 c.c. of the suspension was injected, intramuscularly, in 619. See Clinical Chart below.

NEGATIVE FINDINGS.

1. *Ulcers and Intestinal Mucosa*

The mucosa of the large intestine, and contents of the crypts of the ceca of hogs, contain many spirilla which are evidently constituents of the normal flora. It is also possible that various spirochetes are found in the normal intestinal flora of swine. However, careful dark field examination of material from the intestinal mucosa of a number of hogs not affected with cholera failed to reveal the presence of organisms possessing the characters of *Spirochæta hyos*.

In certain cases where positive findings are made from intestinal lesions, it is necessary to study several specimens in the dark field before *Spirochæta hyos* can be found. More often, in positive cases of hog cholera, the organism can be detected almost instantly in the first microscopical preparation examined. The age and condition of the ulcers appear to have an important bearing on the localization of the spirochete. It has been found with uniformity in young, developing ulcers, while in old, necrotic and healing ulcers it is frequently absent. In the latter condition the spirochete is usually present in the crypts around the ileocecal valve. In conducting the dark field examinations of material from positive cases of hog cholera, usually no difficulty is

experienced in finding *Spirochæta hyos* if one mounts, in a drop of sterile water, a small bit of tissue from the edge of an intestinal ulcer or the contents of a crypt in close proximity to the ileocecal valve.

In attempting to demonstrate *Spirochæta hyos* in the intestinal mucosa, negative findings have resulted from the study of 44 cases. These are shown in table II.

A summary of the above table shows that the organism was not found in the intestinal mucosa in the following cases:

- 31 hogs, susceptible to hog cholera or immune to the disease.
- 3 hogs, typical hog cholera, but treated with toxic doses of mercuric and arsenical preparations.
- 4 hogs, typical hog cholera, but *Spirochæta hyos* found in external local lesions.
- 5 hogs, typical hog cholera. Negative control findings.

In the first group of 31 animals, nine had not been exposed to hog cholera. This affords some valuable data inasmuch as it is difficult to obtain intestinal mucosa from hogs known to be normal and susceptible to hog cholera. We have found that it is practically impossible to obtain such material from packing houses.

Negative findings are recorded from the examination of the ulcers and crypts of eight cases of typical hog cholera. In three of these, however, the spirochete was found in external local lesions, leaving five cases out of the series of forty-four which present negative control findings. Perhaps this may be explained as a discrepancy due to faulty technique or lack of sufficient dark field study of the cases in question. Such an explanation is reasonable when the results from the whole experimental series are considered.

2. External Local Lesions.

The most significant positive findings of *Spirochæta hyos*, in cases of hog cholera, are those from external local lesions. The data offered relative to the presence of *Spirochæta hyos* in local lesions, while not extensive, has an important bearing on the suggestion that this organism may be associated with the etiology of hog cholera. In typical cases of the disease, where local external lesions with necrosis or suppuration have occurred, *Spiro-*

Table II.

Spirochaeta hyos, negative findings in Ulcers and Crypts.

N ^o . of hog	Treatment	Clinical condition	Manner of disposal	Autopsy findings	Condition of animal	Tissues examined by Dark-field method	Nature of results as control observations
612	Culture (VI. 2)	H. cholera, chronic form, full recovery	(VIII. 2) Released, killed and autopsied	Negative	Normal, actively immunized animal	(VIII. 2) Crypts in caecum	
619	Suspension containing spirochaetes and other organisms from cecum of an immune hog A (VI. 27)	Mild reaction, full recovery	idem	idem	idem	idem	
614	Culture (VI. 4)	H. cholera, chronic form, full recovery	"	"	"	"	
621	Filtered (Berkfeld) blood from hog 614 above (VI. 30)	idem	"	"	"	"	
624	Culture (VII. 3)	Reaction, full recovery	"	"	"	"	
625	Culture (VII. 3)	idem	"	"	"	"	
630	Culture (VII. 3). H. C. serum (VII. 3) (VII. 22)	"	"	"	"	"	
671	H. C. virus (X. 3)	Symptoms of H. C. lasting 2 wks. Recovery	(XI. 5) Released, killed and autopsied	Negative, except local lesion at point of inoculation	"	(XI. 5) Sp. hyos found in local lesion (X. 9) and (XI. 5)	
644	Cultur (VIII. 28) Natural exposure (IX. 22).	idem	(XI. 16) idem	Negative	"	(XI. 16) idem	

Immune animals, positive control findings

	No reaction	(IX. 15) Rele- ased and au- topsied	Negative	Normal, acti- vely immu- nized animal	(XI. 16) Sp. hyos found in locallesion (X. 9) and (XI. 5)	Immune ani- mals, positive control fin- dings
669	(IX. 30) (X. 7) Exp. vaccine. H. C. serum (X. 18)	idem	(XI. 16) idem	idem	(XI. 16) idem	
648	(VIII. 25) (IX. 9) Exp. vaccine. H. C. serum (IX. 23)	idem	idem	"	"	
645	Exp. vaccine (VII. 23) (VIII. 15) Natural exposure (IX. 22)	"	"	"	"	
670	H. C. serum (X. 1)	Strong reaction. Symptoms during 1 week. Rapid re- covery	Normal	Immune	(XI. 28) Crypts. Sp. hyos found in ear lesion during reac- tion (X. 8)	
684	Exp. vaccine (X. 20) (X. 27). H. C. serum (XI. 3)	No symptoms of H. cholera. Septi- acemia	No lesions of H. C., blood cultu- res positive. Cocci and bacilli	Hog cholera not present. Septicaemia	(XI. 11) Sp. hyos found in locallesion (X. 9) and (XI. 5)	
702	(XI. 5) H. C. serum	idem	idem	idem	idem	Immune or susceptible animals de- veloped septi- caemia. Posi- tive control findings
699	(XI. 4) (XI. 11) Exp. vaccine. Not exposed	"	"	"	(XI. 13) idem	
697	(XI. 4) (XI. 11) Exp. vaccine. Not exposed	"	"	"	(XI. 16) idem	
700	(XI. 4) (XI. 11) Exp. vaccine. Not exposed	"	"	"	(XI. 15) idem	
457	(XI. 13) (XI. 20) Exp. vaccine. Not exposed	"	"	"	(XI. 24) idem	
727	H. C. serum (XI. 26)	"	"	"	(XI. 28) idem	

No. of hog	Treatment	Clinical condition	Manner of disposal	Autopsy findings	Condition of animal	Tissues examined by Dark-field method	Nature of results as control observations
462	Exp. vaccine (XI. 13). Not exposed	No symptoms of H. cholera. Septicaemia	(XII. 1) Killed and autopsied	No lesions of H. C., blood cultures positive. Cocci and bacilli	Hog cholera not present. Septicaemia	(XII. 1) Sp. hyos found in local lesion (X. 9) and (XI. 5)	
459	Exp. vaccine (XI. 13) (XI. 26). Not exposed	idem	idem	idem	idem	idem	
458	idem						
461	Exp. vaccine (XI. 13) (XI. 20). Not exposed	"	"	"	"	"	
462	(XI. 13). Not exposed	"	"	"	"	"	
693	Exp. vaccine (X. 29) (XI. 7). H. C. serum (XI. 13)	"	"	"	"	"	
726	H. C. serum (XI. 26). Exp. vaccine (XII. 1)	"	(XII. 2) (Died), autopsied	"	"	(XII. 2) idem	
692	(X. 29) (XI. 7) Exp. vaccine (XI. 13). H. C. serum	Reaction, immune	Released, developed septicaemia. Killed and autopsied (XII. 3)	"	"	(XII. 3) idem	
679	Exp. vaccine (X. 11) (X. 25) (XI. 3). H. C. serum (XI. 13)	idem	idem (XII. 6)	"	"	(XII. 6) idem	

Immune or susceptible animals developed septicaemia. Post-mortem control findings

672	Exp. vaccine (X. 6). H. C. serum (X. 27)	Reaction immune	Released, developed septicaemia. Killed and autopsied (XII. 10)	No lesions of H. C., blood cultures positive. Cocci and bacilli	Hog cholera not present. Septicaemia	(XII. 10) Sp. hys found in local lesion (X. 9) and (XI. 5)	
468	Exp. vaccine (XI. 18) (XI. 25). H. C. serum (XII. 4)	No symptoms of cholera. Septicaemia	(Died) autopsied (XII. 12)	No lesions of H. C., blood cultures pos. Cocci and bacilli present. Infested with Ascaroides	idem	(XII. 12) idem	Immune or susceptible animals developed septicaemia. Positive control findings
729	H. C. serum (XI. 26). Exp. vaccine (XII. 1)	idem	idem	idem	"	idem	
596	H. C. serum (IV. 23). Mercury (IV. 28). Atoxyl 1 gm. (V. 2)	H. cholera, acute form and later mercurial poisoning	idem (V. 7)	Lesions of H. C. evidences of chemical poisoning	Hog cholera and chemical caltoxaemia	(V. 7) Ulcers	
607	H. C. serum (Budapest) (V. 14). Arsenical prep. (V. 17)	Marked symptoms of arsenical poisoning	Died 6th day after treatment with arsenical preparation (V. 23)	Possibly some lesions of H. C. No ulcers	Hog cholera (?) and arsenical poisoning	(V. 23) Crypts	Cases of hog cholera, but treated with toxic doses of mercury and arsenic. Positive control findings
608	H. C. serum (V. 14). Neo-salvarsan 0.75 i.v. (V. 20)	idem	Died 4th day after administration of neo-salvarsan (V. 24)	idem	idem	(V. 24) Scrapings from mucosa and crypts	
653	Culture (filtered) (VIII. 27). H. C. serum (IX. 8)	H. cholera, acute form	(Died) autopsied (IX. 29)	Typical lesions of H. C.	Hog cholera, acute form	(IX. 29) Ulcers	Cases of hog cholera. Sp. hys found in local lesions. Positive control findings
657	(IX. 9) H. C. virus	idem	idem (X. 3)	idem	idem	(X. 3) idem	

No. of hog	Treatment	Clinical condition	Manner of disposal	Autopsy findings	Condition of animal	Tissues examined by Dark-field method	Nature of results as control observations
675	H. S. virus (X. 9)	H. cholera, sub-acute form	(Died) autopsied (XI. 2)	Typical lesions. Ulcers	Hog cholera, sub-acute form	(XI. 2) Ulcers and crypts	(Cases of hog cholera. Spir. hyos found in local lesions. Positive control findings)
703	H. C. virus (XI. 6)	H. cholera, acute form	idem (XI. 8)	Some lesions of cholera. No ulcers	Hog cholera	(XI. 18) Crypts	
603	(V. 2) H. S. virus	idem	idem (V. 22)	Lesions of cholera, but no ulcers	Hog cholera, acute form	(V. 22) idem	
639	(VII. 19) (Cultured transfer. 1-557. (IX. 8) H. S. virus	"	idem (IX. 29)	Typical lesions	Hog cholera, sub-acute form	(IX. 29) Ulcers and crypts	
663	Exp. vaccine (IX. 25) (X. 1). H. S. virus (X. 9)	"	idem (X. 31)	Typical cholera	Hog cholera, acute form	(X. 31) idem	Negative control findings
667	Culture (IX. 23). Exp. vaccine (X. 23) (X. 31). H. C. serum (XI. 13)	Symptoms of H. C. and septicaemia	(XI. 24) Killed and autopsied	Slight lesions of cholera and septicaemia. Cocci and bacillus in blood	Hog cholera and Septicaemia	(XI. 24) idem	
745	No treatment	idem	(XII. 12) (Died). Autopsied	Lesions of cholera and septicaemia. Blood cultures positive	idem	(XII. 12) idem	

chæta hyos has been found with uniformity. The experiment is controlled by a series of negative findings.

In the above series of nineteen animals, in which the spirochete was not found in local lesions, the cases are grouped as follows:

- 7 hogs, immune to hog cholera.
- 8 hogs, typical hog cholera. Examination made of bruises or local lesions; tissue not necrotic. *Spirochæta hyos* found in ulcers or crypts.
- 2 hogs, susceptible to hog cholera, not exposed.
- 1 hog, typical hog cholera, *Spirochæta hyos*, not present in fresh abraded surface on ear, but found in same lesion after ten days.
- 1 hog, hog cholera (and septicæmia); *Spirochæta hyos* not found in local lesion or intestinal mucosa. Negative control findings.

In the first of the above groups, consisting of seven immune animals, six hogs (673, "no number," 668, 693, 692 and 691) exhibited lesions, which, according to our experience, probably would have contained *Spirochæta hyos* had the animals been suffering from hog cholera.

Of the whole series, seven animals showed external local tissue abrasions, or areas of skin and subcutaneous tissue necrosis, before they were exposed to hog cholera. The absence of the spirochete in these lesions affords valuable control data.

In one case only, hog 684, which on autopsy showed lesions of hog cholera complicated by septicæmia, the spirochete was found in neither local external lesions nor intestinal mucosa.

In addition to the above data, three cases of hog cholera have been under observation, from the local lesions of which *Spirochæta hyos* has gradually disappeared during convalescence. These results appear in the following clinical charts of hogs 615, 670, and 723.

The data contained in the present investigation represent the results of dark field examinations of material from 237 cases of normal and diseased hogs. The material was obtained from intestinal mucosa, including crypts and ulcers, and local external lesions. The cases consisted of susceptible hogs, those suffering from hog cholera, those immune to the disease, and those affected

Table III.

No. of hog.	Treatment	Clinical conditions	Manner of disposal	Autopsy findings
673	Exp. vaccine (X. 6) H. C. serum (X. 27)	Slight reaction	Released	—
No. number	H. C. serum	idem	(VI. 27) Released, killed and autopsied	Normal
670	H. C. serum (X. 1)	Strong reaction, sick 5 days, rapid recovery	Released	—
668	Exp. vaccine (IX. 30) (X. 7) H. S. virus (X. 18)	Slight reaction	Released (X. 24)	—
693	Exp. vaccine (X. 29) (XI. 7) H. C. virus (XI. 13)	idem	Released. Developed septicaemia, killed and autopsied	(XII. 1). No lesions of H. C. Blood cultures positive. Cocci and bacilli
692	idem	„	idem	(XII. 3) idem
691	„	„	„	(XI. 24) idem
628	Culture (VII. 3) H. C. serum (VII. 27)	Reaction from culture. Chronic cholera from H. C. serum	(Died). Autopsied	(IX. 24). Typical lesions of H. cholera
661	H. C. serum (IX. 23)	H. cholera, acute form	Bled and autopsied	(X. 3) idem
658	(IX. 20) H. S. virus	idem	idem	(X. 14) idem
654	Culture (VIII. 28) Exp. vaccine (IX. 20) H. C. serum (X. 1)	H. cholera, sub-acute form	„	(X. 27) idem
694	H. C. virus (X. 31)	H. cholera, acute form	„	(XI. 13) idem
718	H. C. serum (XI. 26)	H. cholera, acute form	(Died). Autopsied	(XII. 1). Lesions of H. cholera and septicaemia. Also infested with Ascaroides suella
685	Exp. vaccine (X. 20) H. C. serum (XI. 3)	H. cholera and septicaemia	Killed and autopsied	(XI. 15). Lesions of H. C. and septicaemia. Blood cultures positive. Cocci and bacilli
683	idem	idem	idem	idem

Spirochaeta, Negative findings in external local lesions.

Condition of animal	Local lesions examined by Dark-field method	Nature of results as control observations
Normal, actively im- mune animal	(X. 7). Skin lesion 3 cm. in diameter on ventral surface of body	Immune animals. Positive control findings
Normal, natural im- mune	(VI. 27). Skin lesion on right lumbar region	
Normal, actively im- munized animal	(X. 24). Darkened area on mar- gin of ear	
idem	(X. 24). Small purulent exudate around ear tag, right ear. Ma- terial secured on two successive days and examined	Animals immune and suffering from septicaemia. Positive control findings
Immune to H. C. de- veloped septic infec- tion	(XI. 4). Ear, small exudate around tag. (XI. 5). Check exam. ear; also small dry skin lesion on ventral surface	
idem	(XI. 5). Ear, small exudate aro- und tag	
„	(XI. 24). Small exudate around ear tag	Typical cases of hog cho- lera
H. cholera, chronic form	(VIII. 15). Bruised areas just above hoof	
H. cholera, acute form	(X. 2). Slightly abraded right ear under tag. Tissue not nec- rotic	
idem	idem	Spirochaeta hyos found in ulcers and crypts. Positive control findings
H. cholera, subacute form	(X. 27). Small darkened area on margin of ear	
H. cholera, acute form	(XI. 4). Before symptoms. Small, purulent exudate around tag	
H. cholera, septicaemia and infested with as- caroides	(XII. 1). Ear, small abrasion under tag	Animals suffering from hog cholera and septicaemia, Spi- rochaeta hyos found in ulcers and crypts pos. control findings
H. cholera and septi- caemia	(XI. 3). Before exposure to H. C. Small abraded surface under tag, right ear	
idem	idem	

No. of hog.	Treatment	Clinical conditions	Manner of disposal	Autopsy findings
684	Exp. vaccine (X. 20) H. C. serum (XI. 3)	H. cholera and septicaemia	Killed and autopsied	(XI. 15). Lesions of H. C. and septicaemia. Blood cultures positive. Cocci and bacilli
705	Exp. vaccine (XI. 13) (XI. 20). Not exposed to H. C.	(XI. 28). Symptoms of septicaemia	idem	(XII. 1). No lesions of cholera. Blood cultures positive. Cocci and bacilli
731	No treatment. Susceptible animal	Symptoms of septicaemia	„	(XII. 3) idem
675	H. S. virus (X. 9)	H. cholera, acute form	(Died). Autopsied	(XI. 2). Typical lesions of H. cholera

with disease other than hog cholera. In this series of 237 cases, five negative control findings were observed. The results would strongly suggest that *Spirochæta hyos* is practically always present either in the intestinal ulcers or local external lesions of hogs suffering from hog cholera. *Spirochæta hyos* has not been found in normal hogs, but the data are not sufficiently extensive at present to warrant the conclusion that the organism is never present in the normal intestinal mucosa. *Spirochæta hyos* has been found to disappear in the external local lesions of animals convalescent from hog cholera.

The question of filtration of spirochetes is occupying our attention at present. We have already reported successful infection of healthy pigs with hog cholera from the injection of the Berkefeld filtrates of impure cultures of *Spirochæta hyos*.

A recent preliminary publication (12) records the passage of *Spirochæta hyos* through Berkefeld filter N. By this method we have found it possible to secure pure cultures of the organism. The results are in accordance with those published by Dujardin-Beaumetz (13) in the study of the virus of pleuropneumonia, and also by Wolbach and Binger (14), who recently reported the successful cultivation of two filterable spirochetes (*Spirochæta elusa* and *Spirochæta biflexa*), both new species isolated from water.

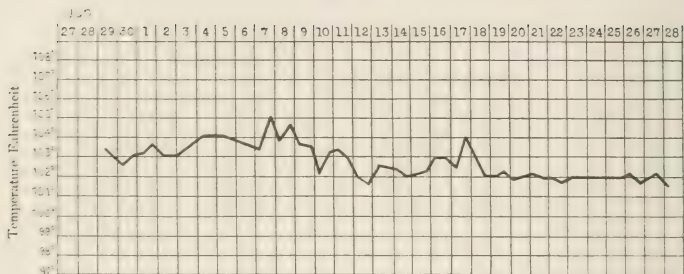
Condition of animal	Local lesions examined by Dark-field method	Nature of results as control observations
H. cholera and septicaemia	(XI. 3). Before exposure to H. C. Small abraded surface under tag, right ear	} <i>Spirochaeta hyos</i> not found in ulcers or crypts of this case. Negative control findings
Septicaemia	(XII. 1). Ear, small abrasion under tag	
idem	(XII. 3). Dry skin lesion containing necrotic tissue on ventral surface of body	} Not exposed in any way to H. cholera. Positive control findings
H. cholera, acute form	(X. 14). Fresh abraded surface on ear, result of fighting	
		} Typical H. cholera, <i>Spirochaeta hyos</i> found in ear lesion (X. 24). Positive control findings

We have made experimental use of salvarsan and neosalvarsan in the treatment of a few cases of hog cholera with negative results; however, it is yet too early in the history of salvarsan to regard it as a specific for all diseases due to spirochetes. As mentioned in a previous report (*Journ. Inf. Dis.*, Vol. 13, 1913, p. 461), we have been able to obtain prolongation of life from the experimental curative treatment of cases of hog cholera with some arsenical and mercurial preparations.

From the findings of Dodd and Cleland, it might be inferred that spirochetes are common invaders of external wounds or abraded skin surfaces occurring in pigs, as these organisms are frequently present in decomposing organic matter with which swine come in contact in their natural environment. This argument does not apply to *Spirochaeta hyos* as we have found it in subcutaneous, unabraded lesions which have never been exposed to external contamination.

On account of the difficulty involved in securing pure cultures of *Spirochaeta hyos*, we have not yet been able to come to any definite conclusion in regard to its pathogenic properties. However, supported by the evidence which we have presented, we may repeat our former conclusion that at the present time "*Spirochaeta hyos* is more nearly established as the specific cause of hog cholera than any other known organism."

Hog No. 619.



June 27. 2 c. c. suspension of material from immune animal.

July 7-9. Slight reaction; indications of malaise, anorexia.

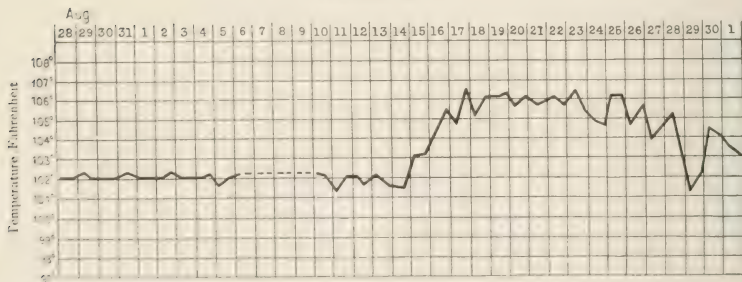
July 16. Animal had been fighting.

July 24. Animal released as normal.

Aug. 2. Animal butchered. All tissues normal. *Spirocheta hyos* not present in crypts of cecum.

Curve 1.

Hog No. 645



Aug. 28. Intramuscular injection 8 c. c. suspension culture filtered, No. 587 (VII. 3. 13) (VIII. 26. 13). (Granular.)

Sept. 16. Anorexia; looks fairly well.

Sept. 17. Appetite poor, feces good, eyes good, looks fair

Sept. 20. Appetite poor, little weak and mild constipated.

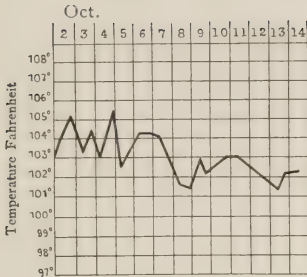
Sept. 22. Appetite better, feces good.

Sept. 29. Anorexia, lungs weak; ear much swollen

Sept. 27. *Spirocheta hyos* present in ear lesion.

Sept. 29. *Spirocheta hyos* present in ear lesion.

Curve 2.

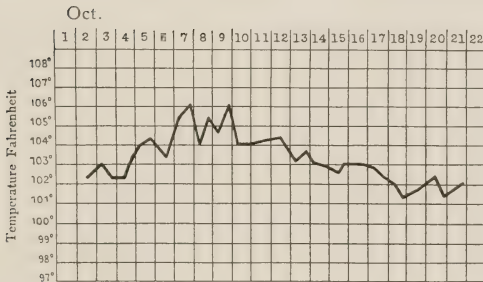


Hog No. 645 (Continued).

- Oct. 2. Very Sick.
 Oct. 9. Seems better.
 Oct. 14. Released as immune.
 Oct. 2. *Spirochæta hyos* present in ear lesion.
 Oct. 8. Few *Spirochæta hyos* present in ear lesion.
 Oct. 24. No *Spirochæta hyos* found in healing ear lesion.

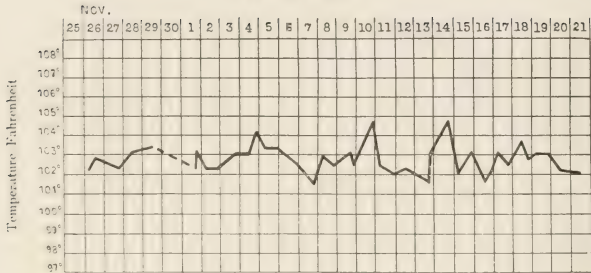
Curve 3.

Hog No. 670.



- Oct. 1. Intramuscular injection 2 c. c. virus 656. Cycle I.
 Oct. 7. Seems a little sick.
 Oct. 10. Weaker, looking gaunt.
 Oct. 13. Eats well, has a chill.
 Oct. 14. Eats fair, chill.
 Oct. 15. Normal.
 Oct. 22. Released as immune.
 Oct. 8. *Spirochæta hyos* present in small lesion, ear, under tag.
 Oct. 24. *Spirochæta hyos* not present in ear lesion, practically healed.

Curve 4.



- Nov. 25. Had been confined for few days with hogs suffering from cholera. Intramuscular injection 1 c. c. Exp. Vac. IV.
- Dec. 1. Very sick, probably chronic cholera; 2 c. c. Exp. Vac. IV.
- Dec. 3. Good appetite.
- Dec. 4. Intramuscular injection 1 c. c. Exp. Vac. IV.
- Dec. 10. Intramuscular injection 3 c. c. Exp. Vac. IV.
- Dec. 11. Condition better.
- Dec. 17. Fine condition; good appetite.
- Dec. 24. Good appetite.
- Dec. 31. Released as immune.
- Dec. 8. Many *Spirochæta hyos* in small purulent exudate on ear, under tag.
- Dec. 19. Very few *Spirochæta hyos* in ear lesion.
- Dec. 29. No Spirochetes in ear lesion.

Curve 5.

ZUSAMMENFASSUNG.

Die in der vorliegenden Arbeit enthaltenen Angaben sind die Resultate von Dunkelfeldprüfungen bei 237 Fällen normaler und kranker Schweine. Das Material stammte von Darmschleimhaut mit Krypten und Geschwüren sowie ausseren lokalen Läsionen. Die Fälle bestanden aus empfänglichen Schweinen, und zwar teils Tieren, die an Schweinepest litten, teils solchen, die gegen die Krankheit immun waren, und endlich solchen, die mit einer anderweitigen Krankheit als Schweinepest befallen waren. In dieser Serie von 237 Fällen wurden 5 negative Kontrollbefunde beobachtet. Die Ergebnisse weisen mit Deutlichkeit darauf hin, dass die *Spirochæta hyos* immer bei an Schweinepest erkrankten

Schweinen zu finden ist, sei es in den Darmgeschwüren oder in den äusseren lokalen Läsionen der Tiere. Die *Spirochæta hyos* wurde in gesunden Schweinen nicht gefunden, jedoch sind die Befunde zurzeit nicht ausgedehnt genug, um den Schluss zu gestatten, dass der Organismus in der normalen Darmschleimhaut nie vorhanden sei. Bei Tieren, die von Schweinepest genasen, ist in den äusseren lokalen Läsionen das Verschwinden der *Spirochæta hyos* festgestellt worden.

Wir kommen zur Frage betreffend die Filtrierung der Spirochäten. Wir haben schon über die erfolgreiche Infektion gesunder Schweine mit Schweinepest berichtet, die nach der Injektion von Berkefeld-Filtraten unreiner Kulturen von *Spirochæta hyos* eintrat. In einer kürzlich erschienenen vorläufigen Mitteilung (12) wird über das Durchtreten der *Spirochæta hyos* durch Berkefeld-Filter N berichtet. Durch diese Methode wurde es uns ermöglicht, den Organismus in Reinkultur zu erhalten. Die Ergebnisse stimmen überein mit denjenigen, die Dujardin-Beaumetz (13) veröffentlichte bei Untersuchungen über das Virus der Pleurpneumonie, ferner Wollach und Bingen (14), welche vor kurzem über die erfolgreiche Züchtung zweier filtrierbarer Spirochäten (*Spirochæta elusa* und *Spirochæta biflexa*) berichteten, die beide neue, aus Wasser isolierte Arten darstellen.

Wir haben bei der Behandlung einiger Fälle von Schweinepest versuchsweise Salvarsan und Neosalvarsan mit negativem Resultate angewandt; jedenfalls stehen wir noch zu sehr im Anfang der Geschichte des Salvarsans, als dass wir die Berechtigung hätten, es für ein Spezifikum bei allen Spirochäten-krankheiten zu halten. Wie in einem früheren Bericht (*Journ. Inf. Dis.*, Vol. 13, 1913, p. 464) erwähnt wurde, gelang es uns, durch die experimentelle Behandlung von Schweinepestfällen mit einigen Arsen- und Quecksilberpräparaten eine Verlängerung des Lebens zu erzielen.

Aus den Befunden von Dodd und Cleland könnte man schliessen, dass Spirochäten gemeine Bewohner äusserer Wunden oder abgeschabter Hautstellen bei Schweinen seien, indem diese Organismen oft in sich zersetzendem organischen Material vorhanden sind, mit dem die Schweine in ihrer natürlichen Umgebung in Berührung kommen. Dieser Schluss lässt sich nicht anwenden auf *Spirochæta hyos*, da wir dieselbe in subkutanen,

nicht offenen Läsionen gefunden haben, welche niemals einer von aussen kommenden Verunreinigung ausgesetzt waren.

In Anbetracht der Schwierigkeiten, die sich der Herstellung von Reinkulturen der *Spirochaeta hyos* entgegenstellen, ist es uns noch nicht gelungen, zu einem abschliessenden Urteil bezüglich ihrer pathogenen Eigenschaften zu gelangen. Jedenfalls möchten wir, gestützt auf die Beweise, die wir erbracht haben, unseren früheren Schluss wiederholen, dass zurzeit „*Spirochaeta hyos* näher als irgend ein anderer bekannter Organismus als die spezifische Ursache der Schweinepest gekennzeichnet ist.“

REFERENCES.

- 1) King and Hoffmann, Journ. Infect. Dis., Vol. 13, 1913, p. 463.
- 2) — and Baeslack, Journ. Infect. Dis., Vol. 12, 1913, p. 307.
- 3) — — and Hoffmann, Journ. Infect. Dis., Vol. 12, 1913, p. 365.
- 4) Arnheim, Zeitschr. f. Hyg. u. Infektionskr., Bd. 76, 1913, p. 407.
- 5) Bosanquet, "Spirochetes", published by the B. Saunders Co. in 1911, p. 95.
- 6) Dodd, Journ. Comp. Path., Vol. 19, 1906, p. 216.
- 7) Cleland, Parasitology, I, 1908, p. 218.
- 8) Neveau-Lemaire, "Parasitology des animaux domestiques", Paris, 1912.
- 9) v. Rütger, Berl. tierärztl. Wochenschr., Bd. 27, 1911, p. 191.
- 10) Uhlenhuth and Haendel, Handb. d. pathog. Mikroorg., Bd. 6, 1913, 1. 303.
- 11) Betegh, Berl. tierärztl. Wochenschr., Bd. 28, 1912, p. 968.
- 12) King and Drake, Journ. Infect. Dis., Vol. 14, 1914, p. 246.
- 13) Dujardin-Beaumetz, Handb. der pathog. Mikroorganismen, Bd. 8, 1913, p. 943.
- 14) Wolbach and Binger, Journ. Med. Research, Vol. 30, 1914, p. 9 and 23.

REPRINTS OF PUBLICATIONS FROM THE RESEARCH
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The present system of collecting reprints of articles published from the Research Laboratory was begun in 1912. Reprints of the following articles published subsequent to that time are available and will be sent upon request. The publications marked (*) are no longer available.

1. On the Administration of Diphtheria Toxin in a Collodion Sac. By E. C. L. Miller. (*Journal of Infectious Diseases*, Vol. 8, January, 1911, pp. 50-65.)

2. A Further Contribution to Our Knowledge of Insecticides—Fumigants. By Chas. T. McClintock, H. C. Hamilton and F. B. Lowe. (*Journal of the American Public Health Association*, Vol. 1, April, 1911, pp. 227-238.)

3. *Duboisia Hopwoodii*—A Histological Study. By Oliver A. Farwell. (Reprinted from *Merck's Report*, Vol. 20, May 1, 1911.)

*4. Etiology of Canine Distemper. By Newell S. Ferry. (*Journal of Infectious Diseases*, Vol. 8, June, 1911, pp. 399-420.)

*5. The Resistance of Smallpox Vaccine to the Coal-tar Disinfectants. By Chas. T. McClintock and Newell S. Ferry. (*Journal of the American Public Health Association*, Vol. 1, June, 1911, pp. 418-419.)

6. Production of Immunity with Over-Neutralized Diphtheria Toxin. By Chas. T. McClintock and Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Originale, Bd. 59, July 15, 1911, pp. 456-464.)

7. Soaps from Different Glycerides—Their Germicidal and Insecticidal Values Alone and Associated with Active Agents. By H. C. Hamilton. (*Journal of Industrial and Engineering Chemistry*, Vol. 3, August, 1911, pp. 582-584.)

*8. The Sleepy Grass of New Mexico: A Histological Study. By Oliver A. Farwell. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-273.)

*9. Some Observations on the Physiological Action of Sleepy Grass. By A. W. Lescohier. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-275.)

*10. An Investigation of the Depressor Action of Pituitary Extracts. By Carey P. McCord. (*Archives of Internal Medicine*, Vol. 8, November, 1911, pp. 609-620.)

11. The Physiology of the Pituitary Gland and the Actions of Its Extracts. By Carl J. Wiggers. (*American Journal of Medical Sciences*, Vol. 141, April, 1911, pp. 502-515.)

12. A Physiological Investigation of the Treatment of Hemoptysis. By Carl J. Wiggers. (*Archives of Internal Medicine*, Vol. 8, 1911, pp. 17-38.)

13. Notes on Catgut Sterilization: A Preliminary Report. By Willard H. Hutchings. (*Annals of Surgery*, Vol. 54, July, 1911, pp. 693-695.)

14. The Relations of Pyogenic Microorganisms to the Etiology and Treatment of Skin Diseases. By Henry Rockwell Varney. (*Ohio State Medical Journal*, December, 1911.)

15. A Micrococcus with Unusual Characteristics as a Factor in a Resistant Dermatitis Resembling Acne Vulgaris. By Henry Rockwell Varney and L. T. Clark. (*Journal of Cutaneous Diseases*, Vol. 30, February, 1912, pp. 72-78.)

16. Serum Treatment of Hemorrhage and Blood Dyscrasias. By A. W. Lescohier. (*New York Medical Journal*, Vol. 95, February 3, 1912, pp. 223-229.)

*17. Further Studies on the Bacillus Bronchicanis, the Cause of Canine Distemper. By Newell S. Ferry. (*American Veterinary Review*, Vol. 41, April, 1912, pp. 77-79.)

18. The Pharmacopoeial Requirements for Cannabis Sativa. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, March, 1912, pp. 200-203.)

19. The Heart Tonic Unit. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 84, March, 1912, pp. 97-103.)

20. Studies on the Etiology of Equine Influenza. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, April, 1912, pp. 185-197.)

21. A Method for the Bacteriological Standardization of Disinfectants. By Tatsuzo Ohno and H. C. Hamilton. (*American Journal of Public Health*, Vol. 2, May, 1912, pp. 331-338.)

22. Physiological Testing. By E. M. Houghton. (*American Druggist*, July and September, 1911, and January and April, 1912.)

23. Bacillus Bronchisepticus (Bronchicanis): The Cause of Distemper in Dogs and a Similar Disease in Other Animals. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, July, 1912, pp. 376-391.)

24. On Feeding Young Pups the Anterior Lobe of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 30, July, 1912, pp. 352-357.)

25. A Practical Portable Incubator. By Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Original, Bd. 65, Heft 4/5, 1912, pp. 412-413.)

26. Tobacco Extracts: Their Comparative Values as Insecticides. By W. O. Hollister. (*Journal of Economic Entomology*, Vol. 5, June, 1912, pp. 263-267.)

27. The Pharmacological Assay of Pituitary Preparations. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, October, 1912, pp. 1117-1119.)

28. Pituitary Extracts in Obstetrics and Gynecology. By A. W. Lescohier and O. E. Closson. (*Journal of the Michigan State Medical Society*, Vol. 11, October, 1912, pp. 650-657.)

29. Biological Products—Veterinary. By Robert H. Wilson. (*American Veterinary Review*, Vol. 41, September, 1912, pp. 668-681.)

30. The Isolation and Cultural Characteristics of Bacillus Acne. By Edwin M. Stanton. (*Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Original, Bd. 66, Heft 5/7, 1912, pp. 386-389.)

31. Studies on Hog Cholera. By Walter E. King and Robert H. Wilson. (*Journal of Infectious Diseases*, Vol. 11, Nov., 1912, pp. 441-458.)

32. Studies on the Virus of Hog Cholera. By Walter E. King and F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 39-41.)

33. The Physiological Activity of Cannabis Sativa. By H. C. Hamilton, A. W. Lescohier and R. A. Perkins. (*Journal of the American Pharmaceutical Association*, Vol. 2, Jan., 1913, pp. 22-30.)

34. The Iodine Content of the Small, Medium and Large Thyroid Glands of Sheep, Beef and Hogs. By T. B. Aldrich. (Original Communications, Eighth International Congress of Applied Chemistry, Vol. 19, 1912, pp. 9-14.)

35. Studies on the Virus of Hog Cholera. By Walter E. King and Robert H. Wilson. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 16, Heft 3, 1913, pp. 367-376.)

36. On the Cultivation of the Treponema Pallidum (*Spirochæta Pallida*). By F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 55-67.)

*37. Studies on the Gonococcus, I. By Carl C. Warden. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 93-105.)

38. Studies on the Virus of Hog Cholera. By Walter E. King, F. W. Baeslack and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 12, March, 1913, pp. 206-235.)

39. Bacillus Bronchisepticus—Its Relation to Canine Distemper. By N. S. Ferry. (*American Veterinary Review*, Vol. 43, April, 1913, pp. 16-30.)

40. Drug Influence on Extrasystoles of the Mammalian Heart. By Carey P. McCord. (*Interstate Medical Journal*, Vol. 19, Oct., 1912, pp. 870-880.)

41. The Employment of Protective Enzymes of the Blood as a Means of Extracorporeal Diagnosis. I.—Sero-Diagnosis of Pregnancy. By Carey P. McCord. (*Surgery, Gynecology and Obstetrics*, Vol. 16, April, 1913, pp. 418-421.)

42. Tribromo-tert-Butyl Alcohol, $C_4H_7OBr_3$. By T. B. Aldrich. (*Journal of the American Chemical Society*, Vol. 33, March, 1911, pp. 386-388.)

43. On Feeding Young White Rats the Posterior and the Anterior Parts of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 31, Nov., 1912, pp. 94-101.)

44. The Rationale of the Use of Adrenalin in the Treatment of Asthma. By Carey P. McCord. (*Medical Record*, Vol. 83, March 8, 1913, pp. 431-432.)

45. Standardization of Disinfectants: Some Suggested Modifications. By H. C. Hamilton and T. Ohno. (*American Journal of Public Health* Vol. 3, June, 1913, pp. 582-588.)

46. Preventive Measures Against Equine Influenza Based on Its Bacteriology. By N. S. Ferry. (Report of the Proceedings of the United States Live Stock Association, December, 1912, p. 127.)

47. Correcting Water. By H. C. Hamilton. (*Bulletin of Pharmacy*, Vol. 27, August, 1913, pp. 330-335.)

48. Duration of Immunity Following Small-pox Vaccination. By A. W. Lescohier. (*Journal of the American Medical Association*, Vol. 61, Aug. 16, 1913, page 487-490.)

49. On Crystalline Kombe-Strophanthin. By D. H. Brauns and O. E. Closson. (*Journal of the American Pharmaceutical Association*, May, June and July, 1913, Vol. 2.)

50. A Comparative Study of Antigens for the Wassermann Reaction. By H. R. Varney and F. W. Baeslack. (*Journal of the American Medical Association*, Vol. 61, Sept. 6, 1913, pp. 754-757.)

51. The Treatment of Tetanus. By Charles T. McClintock and Willard H. Hutchings. (*Journal of Infectious Diseases*, Vol. 13, Sept., 1913, pp. 309-320.)

52. *Spirochæta Suis*, Its Significance as a Pathogenic Organism, Studies on Hog Chlorea. By Walter E. King and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 13, Nov., 1913, pp. 463-498.)

53. Time Recorder for Kymograph Tracings. By Oliver E. Closson. (*Journal of Pharmacology and Experimental Medicine*, Vol. 5, Jan., 1914, pp. 235-238.)

54. U. S. P. Menstrua. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 86, Feb., 1914, pp. 56-61.)

55. Numerical Variations of the White Blood Cells in Mice Inoculated with Transplantable Adenocarcinoma. By F. W. Baeslack. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 20, Heft 5, 1914, pp. 421-435.)

56. A Study of the Germicidal Action of the Ultraviolet Rays. By E. M. Houghton and L. Davis. (*American Journal of Public Health*, Vol. 4, March, 1914, pp. 224-240.)

57. Some Phenomena Involved in the Life History of Spirochæta Suis—Studies on Hog Cholera. By W. E. King and R. H. Drake. (*The Journal of Infectious Diseases*, Vol. 14, March, 1914, pp. 246-250.)

58. The Sterilization of Adrenalin Solutions. By L. W. Rowe. (*American Journal of Pharmacy*, Vol. 86, April, 1914, pp. 145-149.)

59. Infection and Immunity: A Review. By N. S. Ferry, Ph.B., M.D. (*Journal of the American Pharmaceutical Association*, Vol. 3, April and May, 1914.)

60. Disinfection—What Disinfectant is the Most Generally Applicable for Clinical, Surgical and Sanitary Purposes? By H. C. Hamilton. (*Therapeutic Gazette*, Vol. 38, May, 1914, pp. 311-315.)

61. Study of the Bacteriology of the Posterior Nasopharynx in Scarlatina. By N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, pp. 934-935.)

62. Some Experiences with Bacterial Vaccines in Scarlatina. By Guy L. Kiefer, M.D., D.P.H., and N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, p. 936.)

63. A Sero-enzyme Test for Syphilis. By F. W. Baeslack, M.A., M.D. (*The Urologic and Cutaneous Review*, Vol. 18, May, 1914, pp. 234-238.)

64. Bacteriology and Control of Acute Infections in Laboratory Animals. By N. S. Ferry, Ph.B., M.D. (*Journal of Pathology and Bacteriology*, Vol. 18, 1914, pp. 445-455.)

65. The Bacteriological Standardization of Disinfectants. By H. C. Hamilton and Tatsuzo Oono. (*American Journal of Public Health*, Vol. 4, No. 6, p. 163.)

66. The Pineal Gland in Relation to Somatic, Sexual and Mental Development. By Carcy P. McCord, M.D. (*Journal of the American Medical Association*, Vol. 63, July 18, 1914, pp. 232-235.)

67. The Sero-enzyme Test for Syphilis. By F. W. Baeslack, M.D., M.A. (*Journal of the American Medical Association*, Vol. 63, Aug. 15, 1914, pp. 559-563.)

68. A Case of Contagious Broncho-pneumonia Caused by Bacillus Coli Communis. By Edwin M. Stanton. (*American Veterinary Review*, Vol. 14, May, 1914, pp. 233-235.)

69. Local Anesthetics—Some Comparative Physiological Reactions. By Oliver E. Closson. (*Journal of the Michigan State Medical Society*, Vol. 13, Oct., 1914, pp. 587-597.)

70. Potassium Tellurite as an Indicator of Microbial Life. By Walter E. King and Lewis Davis. (*American Journal of Public Health*, Vol. 4, Oct., 1914, pp. 917-931.)

71. Further Studies with Reference to Spirochetes Observed in Swine—Studies on Hog Cholera. By Walter E. King, Raymond H. Drake, and Geo. L. Hoffmann. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Vol. 22, 1914, pp. 347-371.)

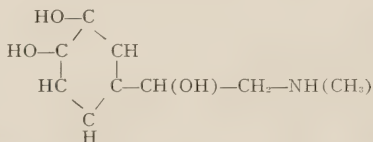
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THE PHARMACY OF ADRENALIN.

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Adrenalin has to-day a well-established place in the *materia medica*. In therapy, its field is large and expanding. Its chemistry and pharmacology have been studied elaborately. Of its pharmacy, however, comparatively little has been written. In dispensing this sensitive substance, there is much opportunity for error. I believe it a moderate estimate that of adrenalin-containing prescriptions met in actual practice, more than half are either ill-written or improperly compounded. In the present paper, it is proposed to discuss briefly the pharmacy of adrenalin, and, particularly, to suggest certain expedients and precautions favoring the conservation of its activity alone and in mixture.

The structural formula, when known, constitutes perhaps the most precise possible definition of a pure chemical compound. In the case of adrenalin, the formula has been established beyond doubt, by both analysis and synthesis; for the sake of precision, therefore, let us define adrenalin as the *lævo*-rotatory isomer of the formula,



The *dextro*-rotatory isomer of the same formula, which has been found in the synthetic product only, is probably nearly or quite inert.

While this formula is before us, it is well to observe that the molecule contains groups that characterize it at once as an amine base, an alcohol and a phenol—an observation that will help to a clearer understanding of its chemical behavior.

Adrenalin occurs naturally in the medulla of the suprarenal gland of vertebrate animals, including man. Suprarenal glands

of oxen, which are most readily obtainable in sufficient amount, supply the adrenalin of commerce. We have no reason to doubt, however, that the natural substance is the same from whatever animal obtained.

Details of a process, identical in all essential points with that used on the manufacturing scale for the isolation of adrenalin from the gland tissue, may be found in an article by Dr. Takamine in the *American Journal of Pharmacy*, November, 1901.

Adrenalin of commerce, a nearly white micro-crystalline powder, is substantially pure, containing a very small fraction only of foreign matter. When properly stored, the pure crystals will remain unchanged for many years or perhaps indefinitely, but certain precautions should be observed as to the conditions of storage. Air, ammonia and certain other gases, moisture, strong light and heat, are in different degrees injurious. Under the combined action of air and moisture, adrenalin is decomposed rapidly. It is well, therefore, to store the product away from strong light and heat, in absolutely dry glass bottles or tubes, sealed air-tight. The drying of the container should be very thorough indeed, and of course the substance itself should be quite dry. A further precaution, superfluous however in ordinary practice, is to displace the air from the container with a dry inert gas. So protected and hermetically sealed, it is probable that adrenalin would prove absolutely permanent.

The pure substance is slightly soluble in cold water, and to a somewhat greater extent in hot water. Other ordinary simple solvents dissolve it very little, if at all, though aqueous solutions of certain salts have a marked solvent action. For instance, a strong aqueous solution of a borate dissolves adrenalin abundantly, and borates prevent its precipitation by alkalis from solutions of its salts. Also, a strong aqueous solution of chemically neutral adrenalin chloride will dissolve an appreciable amount of the adrenalin base.

In virtue of its phenol function it forms water-soluble compounds with fixed caustic alkalis, but not with their carbonates nor with ammonia. Hence, from strong solutions of most of its salts, while the adrenalin base is partly precipitated by hydroxides or carbonates of strong alkalis including ammonium, it is redissolved by excess of the fixed caustic alkalis only.

None of the solutions mentioned so far is recommended for use in pharmacy, since in all the adrenalin is oxidized rapidly on exposure to air.

In virtue of its amine function, adrenalin forms definite salts with the acids, usually very hygroscopic and difficult to preserve in dry form. They are in general very soluble in water and in alcohol, and these solutions may be made sufficiently stable for all ordinary uses. The salts are not very soluble in common simple solvents other than water and alcohol. For most pharmaceutical purposes, therefore, we are limited to the use of the salts of the base, in aqueous or alcoholic solution.

For instance, desiring to make an ointment containing adrenalin, the best practice is to prepare, first, a concentrated aqueous solution of the chloride by dissolving adrenalin in the proper quantity of cold, moderately diluted hydrochloric acid, to incorporate the solution with sufficient lanolin, which as you know will take up much water, and to add, finally, whatever other ointment base may be prescribed. In case the prescribed fatty base is miscible with sufficient alcohol, it may infrequently be advisable to dissolve the adrenalin in the proper proportion of alcoholic, instead of aqueous, hydrochloric acid.

Very occasionally, ointments, suppositories, bougies, etc., containing adrenalin, are ordered, and the general method for ointments just outlined will suggest how they may be prepared.

Most commonly, however, aqueous solutions are prescribed; in fact commercial solution of adrenalin chloride, rather than the adrenalin base, is the usual starting point in prescription compounding. Some discussion is necessary, therefore, of the composition and properties of this preparation and of the precautions necessary to its conservation.

Commercial solution of adrenalin chloride contains one part per thousand of adrenalin chloride dissolved in physiologic salt solution with about one-half per cent of chloretone. Also, it is nearly saturated with carbon dioxide used in the manufacturing process to expel dissolved air. It is faintly acid in reaction, tastes of chloretone and salt, smells of chloretone, and, when fresh, is nearly colorless. Stored away from strong light and heat, with the seal unbroken, the solution will retain its activity for a long period. When, however, the stopper is removed and

contact with air permitted, a new factor is to be considered. The oxygen of the air is destructive of adrenalin. Given good storage, the precaution most essential to the preservation of the commercial solution of adrenalin chloride is to minimize contact with air. Only so much as is required for immediate use should be removed from the stock-bottle, which should be stoppered promptly and tightly. With ordinary care in handling, there is, within reasonable time, no necessity for serious loss through deterioration.

The oxidation that occurs upon undue exposure to air is evidenced by change of color. The solution becomes pink, then red, then brown, and a brown precipitate settles out. This fact is not without practical application, since the color constitutes a rough, but fairly reliable, index of the potency of the solution. Experiments have been made to discover, if possible, some relation between the shade of color and the amount of deterioration. Solutions have been exposed freely to the air, the several changes of color observed, and physiological assays made from time to time. Of course, any quantitative statement based on personal estimate of a shade of color, is of necessity very crudely approximate. Bearing this in mind, however, and limiting the statement strictly to the undiluted commercial solution, we may say that so long as the color is not deeper in shade than what most persons would call pink, the loss of activity is practically negligible. When it becomes red, the loss of activity is quite measurable. It may amount to 10 or 20 per cent of the whole. When brown, with the brown precipitate, the solution should be rejected, though even such solutions often retain considerable activity.

Aside from the physiological assay, which is too complicated for use in the pharmacy, I know of no entirely reliable assay method for adrenalin. Several colorimetric methods have been proposed, but there is none that I dare recommend as wholly accurate and trustworthy. The colors are often fleeting and vary in tint with the nature of the sample. Certain of the proposed reactions, also, are by no means specific for undecomposed adrenalin.

A rough qualitative test, to show the presence of active adrenalin in the commercial solution of adrenalin chloride of the composition already stated, is based on its conduct with ferric chloride. As you are aware, ferric chloride gives striking and

more or less characteristic color reactions with many of the phenols. Catechol, the parent phenol of adrenalin, gives, in dilute aqueous solution with a very little dilute ferric chloride solution, a brilliant green color, which, upon careful addition of very dilute alkali, passes through a series of color changes from bluish-green to purple-red. Under like conditions, adrenalin acts similarly. The catechol nucleus is responsible for this reaction, so that it is not peculiar to adrenalin. If, however, dilute solutions of catechol and adrenalin chloride be treated with a little very dilute ferric chloride solution without subsequent addition of alkali, and if the two solutions be allowed to stand in the air for some minutes, a difference in their behavior will manifest itself. In the case of catechol, the green color persists; in that of adrenalin, it changes slowly to pink or red. While the test is not absolutely final, it is fair to conclude that a commercial solution of adrenalin chloride retains some activity when a sample, highly diluted, gives with a drop of very dilute ferric chloride solution a green color changing soon to pink or red. Many foreign substances interfere with the test, so that it may not be applicable to adrenalin in mixtures.

Having at hand a solution of adrenalin chloride known to be active, it remains to consider the precautions to be observed in dispensing it, alone and in mixture. Certain mixtures are chemically rational and therapeutically useful. If one is proposed, however, of whose feasibility there is doubt, let me counsel conservatism. Where possible, mixtures are best avoided; adrenalin is a sensitive substance, easily changed by many chemical reagents.

Chiefly to be feared, are alkalies and oxidizing agents. Almost any substance that chemically would be classed as an oxidizing agent is more or less injurious to adrenalin. In this category are such substances as oxygen itself, free chlorine, bromine, iodine and their oxy-acids, permanganates, chromates, nitrites, salts of easily reducible metals, etc. *Iron* is extremely troublesome, because of its wide distribution and because a very minute amount will suffice to shorten measurably the life of an adrenalin solution. Traces of iron in other chemicals, in distilled water and even in glassware, are decidedly to be reckoned with. Of course, iron utensils should never be brought in contact with adrenalin.

Alkalies are no doubt destructive directly, but mainly they are pernicious because they very greatly accelerate the destructive action of oxidizing agents. A faintly alkaline solution of adrenalin exposed to the air will lose its activity very quickly. Such solutions are often prescribed, but ought not to be dispensed unless confirmed by the physician after he has been informed of their instability. Every solution of adrenalin that is expected to retain its activity should show a faint acid reaction. I am acquainted with no satisfactory expedient for preparing a stable adrenalin solution that is not slightly acid. Organic acids and weak mineral acids are not very effective unless present in considerable amount. A minute trace of a strong mineral acid—that is to say, a highly dissociated acid—is to be preferred.

In conformity with the last statement, the non-oxidizing acids in reasonable dilution are not injurious to adrenalin. Dilute sulphuric, sulphurous, hydrochloric, phosphoric, boric, salicylic, acetic, tartaric, citric—in fact, most of the acids commonly used in medicine—are harmless. Oxidizing acids are of course objectionable.

Salts of the common alkaloids, of the alkali metals and, broadly, of the light metals generally, are not intrinsically harmful. If, however, their acid radicals are of weak—that is to say, slightly dissociated—acids, they may indirectly diminish the resistance of the adrenalin to oxidation by partial replacement of the trace of free strong acid normally present.

Phenols of the type of carbolic and cresylic acids are harmless. Ordinary camphors, terpenes, and similar bodies are not injurious, save in so far as they may be to a certain extent carriers of oxygen. Most aldehydes of high molecular weight, alcohols and ketones, are probably harmless. Formaldehyde, however, is directly destructive to adrenalin, and the two are wholly incompatible. As little as 1/10 of 1% of formaldehyde added to solution of adrenalin chloride will render it quite inert within a few hours.

In the foregoing statements of incompatibility, exhaustive accuracy is not pretended and doubtless there may be found exceptions. The purpose is merely to characterize certain broad types. An elaborate table of specific incompatibles, even were the data available, is beyond the scope of this paper.

To exemplify the use of these statements, and to emphasize some of the most important points, it will be well to examine and comment upon a few prescriptions. Some of these were submitted for criticism in the regular course of business; some are written arbitrarily to illustrate a particular case. All, however, are such as might be met with in the experience of any pharmacist.

1. \mathcal{R} Solution Adrenalin Chloride.....1 fluidrachm.

Let us begin at the beginning. This prescription requires only that a fluidrachm be dispensed from stock; yet I venture to say that if filled carelessly, the solution will in many instances undergo deterioration far more rapidly than the same solution in the stock-bottle. Certain precautions are recommended that will apply not only to the present case, but to all prescriptions containing adrenalin. Either a glass-stoppered bottle should be used, or else the lower end of the cork should be covered with paraffin or waxed tissue. The vial should be scrupulously clean, and in particular it should be as nearly as possible free from alkali and iron in soluble form. It is advisable, therefore, to wash out all bottles with strong hydrochloric acid followed by much distilled water. This, however, is only a temporary expedient, and the best plan is to select an insoluble glass.

2. \mathcal{R} Sol. of Adrenalin Chloride.....1 volume.
Distilled water or physiologic salt solution. .9 volumes.

All the comments on the last prescription apply equally to this. In addition, one should make sure that the distilled water or physiologic salt solution is free from alkali and iron. It is a good plan, also, to use water or salt solution that has been freshly boiled and cooled. It has already been remarked that the commercial solution of adrenalin chloride is faintly acid—a condition necessary to its stability. Here, this acidity is reduced, by dilution, to 1/10 its original proportion. In many ordinary bottles there is sufficient soluble alkali to neutralize completely this trace of acid, and so to determine the rapid oxidation of the adrenalin. The life of this solution, therefore, would be greatly prolonged by the addition of chemically pure hydrochloric acid, in such proportion that the finished solution contains 1/100 of one per cent of the absolute acid. Care should be taken that the acid

itself is as nearly as possible free from iron, very appreciable amounts of which are present in many lots of so-called chemically pure hydrochloric acid.

Even when quite sterile at the outset, solutions like the one under consideration are liable to contamination in use. To prevent the development of fungus, a mild antiseptic may be added. Saturation with chloroform or chloretone would improve such solutions.

3. R	Adrenalin	¼ grain.
	Cocaine	5 grains.
	Sodium chloride, C. P.	4 grains.
	Boric Acid	10 grains.
	Chloretone	2½ grains.
	Distilled water, sufficient to make	1 fluidounce.

This solution would probably deteriorate fairly rapidly. There should be present some mineral acid stronger than boric. Very suitable would be hydrochloric acid, C. P., in quantity sufficient to saturate the adrenalin and cocaine, and leave an excess of about 1-100 of one per cent of absolute acid in the finished solution. Also, the boric acid should be free from iron—an impurity very common in even the medicinally pure acid.

4. R	Sodium bicarbonate,	
	Sodium borate,	
	Sodium chloride, aa.	2.5 grains.
	Thymol	1/80 grain.
	Sol. Adrenalin Chloride	1 fluidrachm.
	Distilled water, sufficient to make	1 fluidounce.

Nose and throat specialists sometimes order spray-solutions similar to this. Usually they are designed to be slightly alkaline, so that the addition of acid, even if otherwise advisable, would defeat the intention of the prescriber. In the present case, it would be almost useless to add hydrochloric acid, unless in quantity equivalent to the whole of the sodium bicarbonate and sodium borate. This is of course inadmissible. The remedy is, with the consent of the prescriber, to put up the adrenalin solution separately, instructing the patient to add in proper proportion to each dose of the spray-solution immediately before use. If dispensed as it stands, it will become inactive in a very short time.

5. R	Zinc sulphate	0.05 gramme.
	Cocaine hydrochloride	0.2 gramme.
	Adrenalin solution	10 drops.
	Fennel water, B. P., sufficient to make	15 grammes.

This prescription, dispensed by an English pharmacist on the order of an oculist, underwent marked deterioration within a few days. In reply to inquiry as to the cause, certain possibilities were pointed out. First, it is to be noted that the ten drops of adrenalin solution was diluted to about 15 cc., thus reducing the acidity of the adrenalin solution to such an extent that the trace of alkali yielded by most common glass bottles would be fatal. Further, zinc sulphate very often contains iron. This salt and, indeed, each of the ingredients, as well as the bottle itself, should be tested for iron. It is highly probable that one or both of these causes was accountable for the deterioration. The remedy would be to use materials and container as nearly as possible free from iron and alkali, and to add, as already recommended, hydrochloric acid, C. P., up to 1/100 of 1 per cent of the finished solution.

6. R Mercuric chloride..... $\frac{1}{4}$ grain.
 Sol. Adrenalin Chloride.....2 fluidrachms.
 Water, sufficient to make.....1 fluidounce.

When this prescription was compounded by a pharmacist, there appeared almost immediately a slight grey precipitate, the solution becoming red. The precipitation was due, of course, to the reduction of the mercury, and the color to the oxidation of the adrenalin. That the reaction occurred immediately, resulted, perhaps, from the use of slightly alkaline tap-water. Adrenalin chloride and mercuric chloride solution may be mixed without immediate destructive reaction, if there is present a little free hydrochloric acid. Even with this precaution, however, the adrenalin is destroyed within a comparatively short time. This prescription should not be dispensed.

7. R Cocaine hydrochloride.....9 grains.
 Sol. Adrenalin..... $1\frac{1}{2}$ fluidrachms.
 Iodine4 grains.
 Cherry Laurel water, B. P.....2 drachms.
 Glycerin, q. s., to make.....1 fluidounce.

This prescription is copied from the *Pharmaceutical Journal*, Vol. 16, page 484, where it is condemned because of the evident incompatibility between iodine and cocaine hydrochloride. It is further asserted, however, that the formula might be serviceable if the cocaine hydrochloride were omitted—an obvious error since iodine is quickly destructive to adrenalin.

8. R	Adrenalin chloride.....	.6 grains.
	Camphor	1 ounce.
	Phenol	$\frac{1}{2}$ ounce.
	Olive Oil.....	.8 ounces.

This formula is not practicable by reason of the comparative insolubility of adrenalin chloride in the mixture. A physician stated that a very similar prescription was being filled for him regularly. If so, it is probable that an examination of the product he was using would discover either the almost complete absence of adrenalin or the presence of ingredients not mentioned in the formula.

9. R	Solution Adrenalin chloride.....	1 fluidrachm.
	Sodium benzoate.....	1 grain.

The trace of free hydrochloric acid of the adrenalin solution will be largely replaced by the less efficient benzoic acid. The solution will keep fairly well, but will be less resistant to oxidation than the unmodified solution of adrenalin chloride.

To summarize in part—the following considerations are of first importance in dispensing:

Deterioration of adrenalin solutions is usually due to *oxidation*, either by the oxygen of the air or by an added oxidizing agent.

Oxidation is retarded by acids, but accelerated by alkalies. Solutions alkaline in reaction ought not to be dispensed.

In retarding oxidation, a trace of strong acid is, in general, more efficient than the equivalent amount of a weak acid. Solutions acidified with a trace of a weak acid may be dispensed, but will not be very stable. Resistance to oxidation, other things being equal, seems to be a function of the number of hydrogen ions in a unit volume of the solution. The acid should be used, therefore, not in proportion to the amount of adrenalin chloride present, but in proportion to the total volume of the finished solution. One one-hundredth of one per cent of absolute hydrochloric acid is a suitable proportion.

Prescriptions including oxidizing agents should not be dispensed. Iron salts, in particular, are to be avoided. Containers, distilled water, and all materials entering into the prescription should be as nearly as possible free from iron.

Glassware containing much soluble alkali should not be used. Contact with air should be minimized.

REPRINTS OF PUBLICATIONS FROM THE RESEARCH
LABORATORY, PARKE, DAVIS & CO.,
DETROIT, MICH.

The present system of collecting reprints of articles published from the Research Laboratory was begun in 1912. Reprints of the following articles published subsequent to that time are available and will be sent upon request. The publications marked (*) are no longer available.

1. On the Administration of Diphtheria Toxin in a Collodion Sac. By E. C. L. Miller. (*Journal of Infectious Diseases*, Vol. 8, January, 1911, pp. 50-65.)

2. A Further Contribution to Our Knowledge of Insecticides—Fumigants. By Chas. T. McClintock, H. C. Hamilton and F. B. Lowe. (*Journal of the American Public Health Association*, Vol. 1, April, 1911, pp. 227-238.)

3. Duboisia Hopwoodii—A Histological Study. By Oliver A. Farwell. (Reprinted from *Merck's Report*, Vol. 20, May 1, 1911.)

*4. Etiology of Canine Distemper. By Newell S. Ferry. (*Journal of Infectious Diseases*, Vol. 8, June, 1911, pp. 399-420.)

*5. The Resistance of Smallpox Vaccine to the Coal-tar Disinfectants. By Chas. T. McClintock and Newell S. Ferry. (*Journal of the American Public Health Association*, Vol. 1, June, 1911, pp. 418-419.)

6. Production of Immunity with Over-Neutralized Diphtheria Toxin. By Chas. T. McClintock and Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Originale, Bd. 59, July 15, 1911, pp. 456-464.)

7. Soaps from Different Glycerides—Their Germicidal and Insecticidal Values Alone and Associated with Active Agents. By H. C. Hamilton. (*Journal of Industrial and Engineering Chemistry*, Vol. 3, August, 1911, pp. 582-584.)

*8. The Sleepy Grass of New Mexico: A Histological Study. By Oliver A. Farwell. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-273.)

*9. Some Observations on the Physiological Action of Sleepy Grass. By A. W. Lescohier. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-275.)

*10. An Investigation of the Depressor Action of Pituitary Extracts. By Carey P. McCord. (*Archives of Internal Medicine*, Vol. 8, November, 1911, pp. 609-620.)

11. The Physiology of the Pituitary Gland and the Actions of Its Extracts. By Carl J. Wiggers. (*American Journal of Medical Sciences*, Vol. 141, April, 1911, pp. 502-515.)

12. A Physiological Investigation of the Treatment of Hemoptysis. By Carl J. Wiggers. (*Archives of Internal Medicine*, Vol. 8, 1911, pp. 17-38.)

13. Notes on Catgut Sterilization: A Preliminary Report. By Willard H. Hutchings. (*Annals of Surgery*, Vol. 54, July, 1911, pp. 693-695.)

14. The Relations of Pyogenic Microorganisms to the Etiology and Treatment of Skin Diseases. By Henry Rockwell Varney. (*Ohio State Medical Journal*, December, 1911.)

15. A Micrococcus with Unusual Characteristics as a Factor in a Resistant Dermatitis Resembling Acne Vulgaris. By Henry Rockwell Varney and L. T. Clark. (*Journal of Cutaneous Diseases*, Vol. 30, February, 1912, pp. 72-78.)

16. Serum Treatment of Hemorrhage and Blood Dyscrasias. By A. W. Lescohier. (*New York Medical Journal*, Vol. 95, February 3, 1912, pp. 223-229.)

*17. Further Studies on the Bacillus Bronchicanis, the Cause of Canine Distemper. By Newell S. Ferry. (*American Veterinary Review*, Vol. 41, April, 1912, pp. 77-79.)

18. The Pharmacopœial Requirements for Cannabis Sativa. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, March, 1912, pp. 200-203.)

19. The Heart Tonic Unit. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 84, March, 1912, pp. 97-103.)

20. Studies on the Etiology of Equine Influenza. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, April, 1912, pp. 185-197.)

21. A Method for the Bacteriological Standardization of Disinfectants. By Tatsuzo Ohno and H. C. Hamilton. (*American Journal of Public Health*, Vol. 2, May, 1912, pp. 331-338.)

22. Physiological Testing. By E. M. Houghton. (*American Druggist*, July and September, 1911, and January and April, 1912.)

23. Bacillus Bronchisepticus (Bronchicanis): The Cause of Distemper in Dogs and a Similar Disease in Other Animals. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, July, 1912, pp. 376-391.)

24. On Feeding Young Pups the Anterior Lobe of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 30, July, 1912, pp. 352-357.)

25. A Practical Portable Incubator. By Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Original, Bd. 65, Heft 4/5, 1912, pp. 412-413.)

26. Tobacco Extracts: Their Comparative Values as Insecticides. By W. O. Hollister. (*Journal of Economic Entomology*, Vol. 5, June, 1912, pp. 263-267.)

27. The Pharmacological Assay of Pituitary Preparations. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, October, 1912, pp. 1117-1119.)

28. Pituitary Extracts in Obstetrics and Gynecology. By A. W. Lescohier and O. E. Closson. (*Journal of the Michigan State Medical Society*, Vol. 11, October, 1912, pp. 650-657.)

29. Biological Products—Veterinary. By Robert H. Wilson. (*American Veterinary Review*, Vol. 41, September, 1912, pp. 668-681.)

30. The Isolation and Cultural Characteristics of Bacillus Acne. By Edwin M. Stanton. (*Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Original, Bd. 66, Heft 5/7, 1912, pp. 386-389.)

31. Studies on Hog Cholera. By Walter E. King and Robert H. Wilson. (*Journal of Infectious Diseases*, Vol. 11, Nov., 1912, pp. 441-458.)

32. Studies on the Virus of Hog Cholera. By Walter E. King and E. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 39-41.)

33. The Physiological Activity of Cannabis Sativa. By H. C. Hamilton, A. W. Lescohier and R. A. Perkins. (*Journal of the American Pharmaceutical Association*, Vol. 2, Jan., 1913, pp. 22-30.)

34. The Iodine Content of the Small, Medium and Large Thyroid Glands of Sheep, Beef and Hogs. By T. B. Aldrich. (Original Communications, Eighth International Congress of Applied Chemistry, Vol. 19, 1912, pp. 9-14.)

35. Studies on the Virus of Hog Cholera. By Walter E. King and Robert H. Wilson. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 16, Heft 3, 1913, pp. 367-376.)

36. On the Cultivation of the *Treponema Pallidum* (*Spirochæta Pallida*). By F. W. Baeslack. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 55-67.)

*37. Studies on the *Gonococcus*, I. By Carl C. Warden. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 93-105.)

38. Studies on the Virus of Hog Cholera. By Walter E. King, F. W. Baeslack and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 12, March, 1913, pp. 206-235.)

39. *Bacillus Bronchisepticus*—Its Relation to Canine Distemper. By N. S. Ferry. (*American Veterinary Review*, Vol. 43, April, 1913, pp. 16-30.)

40. Drug Influence on Extrasystoles of the Mammalian Heart. By Carey P. McCord. (*Interstate Medical Journal*, Vol. 19, Oct., 1912, pp. 870-880.)

41. The Employment of Protective Enzymes of the Blood as a Means of Extracorporeal Diagnosis. I.—Sero-Diagnosis of Pregnancy. By Carey P. McCord. (*Surgery, Gynecology and Obstetrics*, Vol. 16, April, 1913, pp. 418-421.)

42. Tribromo-tert-Butyl Alcohol, $C_4H_7OBr_3$. By T. B. Aldrich. (*Journal of the American Chemical Society*, Vol. 33, March, 1911, pp. 386-388.)

43. On Feeding Young White Rats the Posterior and the Anterior Parts of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 31, Nov., 1912, pp. 94-101.)

44. The Rationale of the Use of Adrenalin in the Treatment of Asthma. By Carey P. McCord. (*Medical Record*, Vol. 83, March 8, 1913, pp. 431-432.)

45. Standardization of Disinfectants: Some Suggested Modifications. By H. C. Hamilton and T. Ohno. (*American Journal of Public Health* Vol. 3, June, 1913, pp. 582-588.)

46. Preventive Measures Against Equine Influenza Based on Its Bacteriology. By N. S. Ferry. (Report of the Proceedings of the United States Live Stock Association, December, 1912, p. 127.)

47. Correcting Water. By H. C. Hamilton. (*Bulletin of Pharmacy*, Vol. 27, August, 1913, pp. 330-335.)

48. Duration of Immunity Following Small-pox Vaccination. By A. W. Lescohier. (*Journal of the American Medical Association*, Vol. 61, Aug. 16, 1913, page 487-490.)

49. On Crystalline Kombe-Strophanthin. By D. H. Brauns and O. E. Closson. (*Journal of the American Pharmaceutical Association*, May, June and July, 1913, Vol. 2.)

50. A Comparative Study of Antigens for the Wassermann Reaction. By H. R. Varney and F. W. Baeslack. (*Journal of the American Medical Association*, Vol. 61, Sept. 6, 1913, pp. 754-757.)

51. The Treatment of Tetanus. By Charles T. McClintock and Willard H. Hutchings. (*Journal of Infectious Diseases*, Vol. 13, Sept., 1913, pp. 309-320.)

52. *Spirochæta Suis*, Its Significance as a Pathogenic Organism, Studies on Hog Chlorea. By Walter E. King and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 13, Nov., 1913, pp. 463-498.)

53. Time Recorder for Kymograph Tracings. By Oliver E. Closson. (*Journal of Pharmacology and Experimental Medicine*, Vol. 5, Jan., 1914, pp. 235-238.)

54. U. S. P. Menstrua. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 86, Feb., 1914, pp. 56-61.)

55. Numerical Variations of the White Blood Cells in Mice Inoculated with Transplantable Adenocarcinoma. By F. W. Baeslack. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 20, Heft 5, 1914, pp. 421-435.)

56. A Study of the Germicidal Action of the Ultraviolet Rays. By E. M. Houghton and L. Davis. (*American Journal of Public Health*, Vol. 4, March, 1914, pp. 224-240.)

57. Some Phenomena Involved in the Life History of Spirochaeta Suis—Studies on Hog Cholera. By W. E. King and R. H. Drake. (*The Journal of Infectious Diseases*, Vol. 14, March, 1914, pp. 246-250.)

58. The Sterilization of Adrenalin Solutions. By L. W. Rowe. (*American Journal of Pharmacy*, Vol. 86, April, 1914, pp. 145-149.)

59. Infection and Immunity: A Review. By N. S. Ferry, Ph.B., M.D. (*Journal of the American Pharmaceutical Association*, Vol. 3, April and May, 1914.)

60. Disinfection—What Disinfectant is the Most Generally Applicable for Clinical, Surgical and Sanitary Purposes? By H. C. Hamilton. (*Therapeutic Gazette*, Vol. 38, May, 1914, pp. 311-315.)

61. Study of the Bacteriology of the Posterior Nasopharynx in Scarlatina. By N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, pp. 934-935.)

62. Some Experiences with Bacterial Vaccines in Scarlatina. By Guy L. Kiefer, M.D., D.P.H., and N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, p. 936.)

63. A Sero-enzyme Test for Syphilis. By F. W. Baeslack, M.A., M.D. (*The Urologic and Cutaneous Review*, Vol. 18, May, 1914, pp. 234-238.)

64. Bacteriology and Control of Acute Infections in Laboratory Animals. By N. S. Ferry, Ph.B., M.D. (*Journal of Pathology and Bacteriology*, Vol. 18, 1914, pp. 445-455.)

65. The Bacteriological Standardization of Disinfectants. By H. C. Hamilton and Tatsuzo Ohno. (*American Journal of Public Health*, Vol. 4, No. 6, p. 163.)

66. The Pineal Gland in Relation to Somatic, Sexual and Mental Development. By Carey P. McCord, M.D. (*Journal of the American Medical Association*, Vol. 63, July 18, 1914, pp. 232-235.)

67. The Sero-enzyme Test for Syphilis. By F. W. Baeslack, M.D., M.A. (*Journal of the American Medical Association*, Vol. 63, Aug. 15, 1914, pp. 559-563.)

68. A Case of Contagious Broncho-pneumonia Caused by Bacillus Coli Communis. By Edwin M. Stanton. (*American Veterinary Review*, Vol. 14, May, 1914, pp. 233-235.)

69. Local Anesthetics—Some Comparative Physiological Reactions. By Oliver E. Closson. (*Journal of the Michigan State Medical Society*, Vol. 13, Oct., 1914, pp. 587-597.)

70. Potassium Tellurite as an Indicator of Microbial Life. By Walter E. King and Lewis Davis. (*American Journal of Public Health*, Vol. 4, Oct., 1914, pp. 917-933.)

71. Further Studies with Reference to Spirochetes Observed in Swine —Studies on Hog Cholera. By Walter E. King, Raymond H. Drake, and Geo. L. Hoffmann. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Vol. 22, 1914, pp. 347-371.)

72. The Pharmacy of Adrenalin. By C. P. Beckwith. (*Journal of the American Pharmaceutical Association*, Vol. 3, November, 1914, pp. 1547-1554.)

A Study of the "Tellurite Reaction" with the Colon-Typhoid Group and other Organisms.*

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With 1 Plate.

INTRODUCTION.

In a previous paper on "Potassium Tellurite as an Indicator of Microbial Life,"† by W. E. King and the writer, attention was called to apparent differences in the action of the tellurite towards *B. coli communis* and towards *B. typhosus*, which seemed to warrant further study. As stated at that time, not only did the typhoid bacillus seem to be peculiarly sensitive to the antiseptic action of potassium tellurite, but the results obtained differed materially among various strains. With the cultures then used, the colon bacillus gave a strong reaction even when the concentration of tellurite was one part in 5000, while with *B. typhosus*, very little action occurred with amounts of the salt stronger than 1:50,000.

The present study has been extended to include the action of potassium tellurite on the more important members of the so-called "Colon-Typhoid" group, and also a few other bacterial species. The experimental work was carried out with the view of determining the following:

- a. Difference in the antiseptic action of potassium tellurite on the various members of the group.
- b. Variations in the macroscopical appearance, character and velocity of the "tellurite reaction" under optimum conditions.
- c. The effect of treatment with tellurite as regards the biochemical activities of the organism.

Altogether, twenty different bacterial species were investigated, including some of the more uncommon types, and several not belonging to the Colon-Typhoid group, but closely related to it biochemically. The organisms have been arranged in the same order as they were studied, rather than according to any definite classification.

* Read before the Society of American Bacteriologists, Montreal, P. Q., Dec., 1913.

† Read before the Laboratory Section, American Public Health Assoc., Colorado Springs, Col., Sept., 1913.

EXPERIMENTAL DATA.

A. The Antiseptic Action of Potassium Tellurite.

In determining the antiseptic action of the tellurite on the various bacteria, essentially the same technique was employed as in the previous work. Briefly, this consisted in adding aseptically to plain bouillon in tubes sufficient potassium tellurite to give concentrations of the salt ranging from 1:1000 to 1:300,000. Triplicate tubes at each concentration were then inoculated with 0.1 c.c. of a well-shaken culture of the organism to be studied, grown for 48 hours in plain bouillon. These, together with "control" tubes, containing no tellurite, were incubated for at least 96 hours at 37° C. before recording results. Where "check" results failed to give agreement, additional sets were run until confirmatory tests were obtained.

1. *B. coli communis*.

Five different strains of this organism were examined, including one isolated from a tubercular hip and one from a case of osteomyelitis, the remainder being from urine and faeces. With one strain (\pm 0129 isolated from tubercular hip) a concentration of 1:2500 of potassium tellurite failed to prevent growth and reduction, while the remaining strains gave practically no growth with amounts stronger than 1:5000. At the latter concentration, there was heavy growth and strong reduction of the tellurite in 24 hours with every one of the five strains studied. It would seem, then, that with the colon bacillus as ordinarily encountered, potassium tellurite acts as an antiseptic in amounts stronger than 1:5000.

2. *Bact. (lactis) aërogenes*.

Both of the cultures of this bacterium gave very little growth at 1:5000, but heavy reduction at 1:10,000 and weaker in less than 24 hours. It is probable that the antiseptic action of tellurite on this organism begins with concentrations greater than about 1:5000.

3. *B. capsulatus*.

The two strains of this organism gave a moderate growth at 1:2500 and above, but practically none at 1:1000. Heavy growth appeared in less than 24 hours at 1:20,000 and weaker. Between 1:1000 and 1:2500 seems to be the limit for the antiseptic action on *B. capsulatus*.

4. *B. pneumoniae* (Friedländer).

The pneumobacillus of Friedländer, in spite of its being capsulated as were the two preceding organisms, showed great susceptibility to the antiseptic action of tellurite. Both of the strains gave practically no growth or reduction with concentrations of potassium tellurite stronger than 1:40,000. Even at 1:300,000 growth was very slow and apparent only after 48 hours' incubation. It is very probable that 1:30,000 of tellurite acts antiseptically with this organism.

5. *B. acidi lactici*.

Three strains of the lactic acid bacillus were studied, all of which were obtained from milk. Two gave practically no growth with amounts of the salt stronger than 1:30,000, while the other strain gave a feeble reaction at 1:20,000. The "control" tubes in all three cases showed rather a light growth so that only a moderate reduction of the tellurite could be expected where vegetation was permitted. Apparently the tellurite begins to act as an antiseptic at 1:20,000 with this organism.

6. *B. rhinoscleromatis*.

About 1:30,000 seems to be the limiting antiseptic concentration of tellurite for this organism, no growth occurring with greater amounts of the salt. The growth at 1:30,000 is very slow and apparent only after 96 hours' incubation.

7. *Paracolon Bacillus*.

Both strains of the paracolon bacillus used were obtained from feces, but from widely divergent localities. One strain gave practically no growth with concentrations stronger than 1:30,000, while the other gave a moderate growth at 1:30,000 but none at 1:10,000. In all probability, 1:20,000 is the limiting antiseptic concentration.

8. *B. enteritidis*.

Between 1:10,000 and 1:20,000 of potassium tellurite seems to be the concentration necessary to exert an antiseptic action with this bacterial race. Three strains were examined, one of which—a subculture of the original organism isolated by Gaertner—seemed to be more resistant than the other two, giving growth and reduction at 1:20,000 while the others did not.

9. *B. cholerae suis*.

All three cultures of the bacillus of hog-cholera, so-called, gave no growth with concentrations of tellurite stronger than 1

part in 20,000. Up to 1:50,000 the growth and reduction were very slow and apparent only after 72 hours' incubation.

10. *Bacillus* of swine plague.

This organism which, strictly speaking, belongs to the Hemorrhagic Septicæmia group, has been included by some in the Colon-Typhoid group. Both strains of the bacillus showed practically the same resistance to potassium tellurite. About 1:10,000 seems to be the strength at which antiseptic action first appears. With one strain, concentrations up to 1:50,000 gave a very peculiar, amorphous, black sediment which was rather heavy, although slow in making its appearance.

11. *B. paratyphosus* "A."

Concentrations greater than 1:20,000 had an antiseptic action on both of the strains of *B. paratyphosus* "A" which were studied. In fact, heavy growth and reduction were obtained only with 1:50,000 and weaker. The action is rather slow and appears only after 48 hours' incubation.

12. *B. paratyphosus* "B."

The two strains of this organism appeared to be more resistant to potassium tellurite than *B. paratyphosus* "A." Growth and reduction occurred at 1:10,000 of tellurite with this type as compared with feeble growth at 1:20,000 with type "A." The control cultures with no tellurite showed apparently the same growth as type "A."

13. *B. icteroides*.

Very little growth and reduction were obtained with *B. icteroides* using concentrations of potassium tellurite stronger than 1:50,000. At this latter strength, heavy growth and a typical reaction were obtained, so that in all probability the antiseptic value lies between 1:10,000 and 1:50,000.

14. *B. Zopfii*.

Check results with this organism showed it to be very susceptible to the action of tellurite. Practically no growth occurred at 1:100,000 and stronger, and only very little with one part of potassium tellurite in 300,000 parts of bouillon even after 96 hours' incubation. The control tubes, however, gave a heavy, typical growth.

15. *B. typhosus*.

Five strains of the typhoid bacillus were studied, including two from the United States Hygienic Laboratory. As noted in



The Action of Potassium Tellurite (1:20,000) on some Intestinal Bacteria

the previous work with this organism, there was quite a variation among the different strains. About 1:20,000 seemed to be the antiseptic concentration for three of the strains, while one (\pm 9199) gave growth at 1:10,000 and the other (Hopkins) at first only at 1:50,000 and weaker.

Transplantations were made from some of the tubes containing the Hopkins strain and tellurite 1:50,000 into fresh, plain bouillon. These cultures were incubated for the usual 48 hours and 0.1 c.c. of the resultant suspension added to other bouillon containing the various concentrations of tellurite. Growth now occurred at 1:20,000. By successive treatments as above, it was finally found possible to grow this apparently weak strain of *B. typhosus* in a bouillon containing potassium tellurite 1:2500.

It would seem, then, that as ordinarily met with, one part of tellurite in 20,000 parts of bouillon or other suitable diluent acts as an antiseptic on *B. typhosus*. Some strains may be encountered which are more sensitive. However, as true with some other antiseptics, it is possible, by cultivation in media containing tellurite, and successive transplantations to make the typhoid bacillus so resistant to this salt that it can be grown in concentrations that ordinarily act as germicides. Similar experimentation gave parallel results with the colon and other bacilli, so that the above facts, in all probability, hold true for the action of potassium tellurite on micro-organisms in general.

16. *Bact. dysenteriae* (Shiga).

17. *Bact. dysenteriae* (Flexner).

Two strains of each of the above bacteria were tested, both of which appeared sensitive to the action of tellurite. About 1:10,000 seems to act as an antiseptic with the Shiga type, while the Flexner type gives practically no growth at 1:50,000. In all cases where growth and reduction did occur, it was not apparent until after 72 hours' incubation. The Shiga bacterium would seem to be more resistant to potassium tellurite than the Flexner organism.

18. *B. proteus vulgaris*.

The *Bacillus proteus vulgaris* is fairly resistant to potassium tellurite, the two strains studied showing heavy growth and reduction at 1:10,000, but not at 1:5000. Where growth did occur, it was rapid and occurred in less than 24 hours' incubation.

19. *B. cloacae*.

One strain of this organism was studied, isolated from an abdominal fistula. It showed even greater resistance to potassium tellurite than did *B. proteus vulgaris*, as heavy growth occurred at 1:5000 and weaker in less than 24 hours.

A review of the preceding data shows that, arranged according to the antiseptic action of potassium tellurite on them, the organisms studied would appear as shown in Table I. From this it is seen that the capsulated bacteria—as *B. (mucosus) capsulatus*—are the most resistant, and the *Bacillus Zopfii* the most susceptible to the antiseptic action of potassium tellurite.

TABLE I.

ANTISEPTIC ACTION OF POTASSIUM TELLURITE ON BACTERIA OF THE COLON-TYPHOID TYPHOID GROUP AND ALLIED ORGANISMS.

Organism.	No. of Strains Examined.	Antiseptic Concentration of Potassium Tellurite.
1. <i>B. capsulatus</i>	2	Bet. 1:1000—1:2500
2. <i>B. coli communis</i>	5	About 1:5000
3. <i>Bact. (lactis) aërogenes</i>	2	" 1:5000
4. <i>B. cloacae</i>	1	" 1:5000
5. <i>B. proteus vulgaris</i>	2	Bet. 1:5000—1:10,000
6. <i>B. paratyphosus "B"</i>	2	About 1:10,000
7. <i>B. of swine plague</i>	2	" 1:10,000
8. <i>B. enteritidis</i>	3	Bet. 1:10,000—1:20,000
9. <i>B. typhosus</i>	5	About 1:20,000
10. <i>B. paratyphosus "A"</i>	2	" 1:20,000
11. <i>Paracolon bacillus</i>	2	" 1:20,000
12. <i>B. acidi lactici</i>	3	" 1:20,000
13. <i>B. cholerae suis</i>	3	" 1:20,000
14. <i>B. rhinoscleromatis</i>	1	" 1:30,000
15. <i>B. pneumoniae</i>	2	" 1:30,000
16. <i>Bact. dysenteriae (Shiga)</i>	2	" 1:40,000
17. <i>B. icteroides</i>	2	Bet. 1:40,000—1:50,000
18. <i>Bact. dysent. (Flexner)</i>	2	About 1:50,000
19. <i>B. Zopfii</i>	1	" 1:100,000

Considering the principal organism of the group, it is found that the colon bacillus and the closely allied *Bact. (lactis) aërogenes* show the strongest resistance; *B. enteritidis*, paracolon bacillus and the paratyphoid bacilli would come next, the typhoid bacillus after these, and the dysentery bacteria the last. Theoretically, then, it would seem that a medium containing potassium tellurite could be devised analogous to that proposed by Conradi and Troch* for the diagnosis of diphtheria, which would be of service for differentiation between the colon, typhoid and dysentery organisms. Studies along this line would undoubtedly prove of value.

*G. Wagner, München, med. Wochenschr., 1913, p. 457. Schürmann, W., u. Hajos, E., Deutsch. med. Wochenschr., 1913, p. 186.

B. The "Tellurite Reaction" with the Colon-Typhoid Group and Allied Organisms Under Optimum Conditions.

It was readily apparent that for studying the action of potassium tellurite on bacteria under optimum conditions, the organisms would have to be in a state of active metabolism. Accordingly, the procedure adopted was to take a 24-hour culture of each strain, grown for two generations in 9 c.c. of plain bouillon. To this was added without subsequent mixing 1.0 c.c. of aseptic solutions of potassium tellurite giving final strengths ranging from one part of tellurite in 2500 parts of bouillon to one part in 300,000.

The usual number of "check" and "control" tests were run, using the same bacterial strains and the same number of tubes as in the preceding study of the antiseptic action. Results were noted after half an hour, after fifteen hours, and finally after 96 hours of incubation at 37° C. The 30-minute results were taken at room temperature, but if no action took place at the end of this period, a second observation was made after three hours at 37° C. The various types investigated have been arranged in accordance with Table I.

1. *B. (mucosus) capsulatus*.

This organism gave a distinct brown ring within 15 minutes after the addition of the tellurite. As would be supposed, it was strongest at 1:2500, but still apparent at 1:40,000. After 96 hours, the strongest concentrations showed a thick, stringy, black sediment with the supernatant liquid nearly black. With increasing dilution, the color gradually becomes lighter, so that there is very little reaction at 1:100,000 and none at 1:300,000.

2. *B. coli communis*.

The reaction with the colon bacillus, especially in the stronger concentrations, was extremely rapid, being apparent either as a ring or darkening within several minutes after the addition of the tellurite solution. After half an hour, there was a distinct darkening with all of the strains studied at dilutions as high as 1:100,000. The reduction is practically complete in 24 hours, and is shown by the entire tube being thick and black with 1:10,000 of tellurite and stronger. The reaction at 1:20,000 is characteristic, and appears as a black, clumpy sediment, with the supernatant

bouillon brown and turbid. (Plate 1a.) Reduction is apparent even at 1:300,000.

3. *Bact. (lactis) aërogenes*.

The two strains of this bacterium which were studied showed a rapid and vigorous reaction with potassium tellurite. A distinct darkening was obtained in fifteen minutes with all dilutions up to 1:100,000. The reaction is complete in less than 24 hours, and appears as a black, viscous sediment with dark brown supernatant liquid, growing lighter toward the top. The reduction grows weaker with decreasing concentration, no reaction being apparent at 1:300,000.

4. *B. cloacæ*.

5. *B. proteus vulgaris*.

Both of the above organisms gave practically the same "tellurite reaction." Reduction took place at all concentrations with *B. proteus vulgaris* and up to 1:100,000 with *B. cloacæ*, being apparent within half an hour, either as a ring or darkening. The final reaction appeared as a heavy, flaky, black sediment with the liquid above very dark brown, nearly black. There was the usual decrease of color with the dilution, the typical reaction appearing at 1:30,000 as uniformly yellowish-brown and turbid.

6. *B. paratyphosus* "B."

A strong ring or darkening was obtained within fifteen minutes with both strains of this bacillus in all concentrations up to 1:100,000. The complete reaction is shown by a compact, black sediment with the supernatant liquid very dark brown in strengths of tellurite up to 1:30,000. At the latter concentration and weaker, the sediment is yellow, while the liquid is nearly clear and brown. (Plate 1c.) The reaction at 1:100,000 is very faint.

7. *Bacillus* of swine plague.

A faint darkening was apparent in half an hour with one strain of this organism and in three hours with the other strain. The reduction is not as vigorous as with the preceding types, and appears finally as a compact black sediment with a supernatant, brown, liquid zone which grows clearer and lighter colored toward the top in concentrations up to 1:10,000. From 1:30,000 on, the liquid is uniformly turbid and brown, while the sediment is colored gray. No reduction takes place at 1:300,000.

8. *B. enteritidis*.

All three strains of *B. enteritidis* showed either a brown ring

or darkening in half an hour up to 1:100,000. The reaction is rather vigorous in the concentrations stronger than 1:10,000, the entire tube being almost a uniform greyish-black. At 1:20,000 and weaker, there is a thick, brown to black sediment with the liquid yellowish brown and only slightly turbid. (Plate I c.) The reduction at 1:100,000 is very weak.

9. *B. typhosus*.

As noted in the previous experimentation with this organism, there was some variation in the final appearance of the reaction among the different strains investigated. All, however, gave either a ring or darkening in concentrations including 1:50,000 within 30 minutes. The reduction is much slower than with *B. coli communis* and while rather heavy in concentrations greater than 1:5000, the tubes from 1:10,000 on show a brownish sediment with the liquid above light brown in color and moderately turbid. (Plate I b.) Although both the typhoid and colon bacilli appear brown colored and turbid in dilutions of 1:20,000 and greater, the macroscopical appearance of the tubes, particularly at this strength, differs materially with each organism and can be readily distinguished by one at all familiar with the reaction.

10. *B. paratyphosus* "A."

With concentrations of tellurite up to 1:20,000 and stronger, the reaction is somewhat similar to that obtained with *B. paratyphosus* "B"—a heavy black sediment with the supernatant liquid very dark brown and turbid. With 1:20,000 and weaker, it appears as a heavy yellowish-brown sediment with black specks, while the bouillon is colored a light brown and is slightly turbid (Plate I d). Reduction is apparent at 1:100,000, but not at 1:300,000. Both strains of the organism showed a darkening within half an hour in all strengths up to 1:100,000.

11. *Paracolon Bacillus*.

The reaction with the paracolon bacillus is very similar to that obtained with *B. paratyphosus* "A." In the stronger concentrations, a ring appeared in about fifteen minutes, and a darkening was apparent up to 1:100,000 in half an hour. Complete reaction takes place in about 48 hours and is shown in the amounts stronger than 1:20,000 by a heavy, black sediment with a dark brown liquid layer above and a lighter zone near the top. At

1:30,000 and weaker, the sediment is yellow with black specks, while the supernatant liquid is uniformly light brown and turbid.

11. *B. acidi lactici*.

Compared with the preceding organisms, *B. acidi lactici* is much slower in its reaction with potassium tellurite. Only one strain showed darkening within half an hour, but all three strains studied gave distinct reduction in fifteen hours. The stronger concentrations particularly (1:2500—1:5000) apparently exert a germicidal action, as reduction seemed stronger from 1:30,000 on than with the greater strengths. The typical appearance is a moderately heavy, black, powdery sediment with the liquid above browned and usually clear. There is very little reaction at 1:100,000.

13. *B. cholerae suis*.

All three strains of this bacillus showed darkening or rings with concentrations up to 1:100,000 in half an hour. At 1:10,000 and stronger, the tubes appeared nearly black, while at 1:20,000 and weaker, the tubes were uniformly brown and turbid, but growing lighter colored as the dilution increased. Only one strain gave any reduction at 1:300,000.

14. *B. rhinoscleromatis*.

15. *B. pneumoniæ* (of Friedländer).

Both of the above seem to react slowly with tellurite, very little reduction occurring before fifteen hours. The reaction is quite similar in both cases but more vigorous with the bacillus of Friedländer than with *B. rhinoscleromatis*. In strengths of 1:10,000 and stronger, the tubes show a flaky sediment with the supernatant bouillon nearly clear but browned. At 1:20,000 and dilutions greater, the color grows successively lighter, until at 1:100,000 there is practically no reaction with either organism.

16. *Bact. dysenteriae* (Shiga).

The reaction with this bacterium did not begin to appear before three hours in the stronger concentrations while with the weaker strengths of tellurite, it was not apparent before fifteen hours. The final appearance of the tubes somewhat resembled those obtained with the Friedländer's bacillus, showing a black, powdery sediment and the liquid above browned but nearly clear. At 1:10,000 and weaker, the brown coloration and sediment began to decrease in intensity, so that at 1:100,000 there was very little indication of any action.

17. *B. icteroides*.

Only a slight darkening occurred with both strains of *B. icteroides* in fifteen hours. As with *B. lactici acidii*, there is a heavier reduction at 1:30,000 and weaker than with greater concentrations of tellurite. Macroscopically, the reaction appears as a powdery black or dark brown sediment with a slightly turbid, brown supernatant liquid. No reduction was apparent at 1:300,000.

18. *Bact. dysenteriae* (Flexner).

Contrary to expectations, the Flexner strain of dysentery bacterium gave a stronger reaction with tellurite than did the Shiga strain. All concentrations, including 1:100,000, showed either a ring or darkening in half an hour. At 1:10,000 and stronger, the tubes showed a compact, black sediment with a dark brown, clouded zone above while the remainder of the liquid was clear but browned. With 1:20,000 and weaker, the sediment was brown, and the whole liquid was nearly clear and colored a yellowish brown. No reaction was apparent at 1:30,000.

19. *B. Zopfii*.

Comparable with the weak resistance displayed in the anti-septic tests, *B. Zopfii* showed less reaction with potassium tellurite than any of the preceding organisms. The reduction at 1:50,000 was nearly as intense as at 1:2500, and was apparent in all cases only after 24 hours. The completed reaction appeared as a small, powdery black sediment with the supernatant liquid clear and only slightly darkened. Practically no reduction of the tellurite took place at 1:100,000 and weaker.

From the foregoing, the close relationship between the capacity of an organism for reducing potassium tellurite and its resistance to the antiseptic action of the latter salt is quite apparent. Those bacteria which require a relatively strong concentration of tellurite (1:500 or more) to inhibit their growth are found to give a rapid and vigorous reaction, while the sensitive types require a much longer period, and the amount of reduction is small.

Not only is the intensity of the "tellurite reaction" dependent upon the reacting organism, but is directly influenced by the amount of the salt present. As would naturally be supposed, for any specific microbe, the greater the amount of potassium tellurite present which can be acted upon, the more will be the extent of the reduction. The "modus operandi," so to speak, can be

conceived to be as follows. Assuming an organism to be in an active state of metabolism and adding to it a strong "dose" of tellurite, two opposing tendencies come into play. On the one hand, there is the "positive" tendency toward continuance of growth and consequent reduction of the potassium tellurite, while on the other hand, there is the "negative" influence tending to inhibit the metabolism of the organism. If the organism be sensitive, the resultant effect will be more reduction with smaller amounts of tellurite present than with greater. This was actually found to be the case with *B. acidi lactici*, *B. Zopfii*, and *B. rhinoscleromatis*. In fact, with *B. Zopfii*, which is very sensitive towards potassium tellurite, concentrations of 1:5000 and stronger permit a reduction at first, but the reaction soon ceases owing to the stronger antiseptic effect assuming the mastery. This is analogous to the findings of Belfanti,* who obtained a black spot in less than four hours with the typhoid bacillus and the strongly germicidal concentration of 1 per cent of potassium tellurite.

Where the two influences above mentioned are nearly equal, the intensity of the reaction will be about the same with all concentrations, except the very weak ones. Such a behavior is displayed by the dysentery bacteria and Friedländer's bacillus. Finally, there is the condition obtained with the more common members of the "Colon-Typhoid" group where the organism is fairly resistant to the antiseptic action of potassium tellurite. In this case, the action quickly begins to take place, is very intense in the stronger concentrations, and appears to be completed within twenty-four hours.

Apparently, the velocity with which potassium tellurite is reduced is a specific function of the reacting organism apart from its resistance to the antiseptic action of the salt, and is dependent upon the concentration of tellurite present only within very broad limits. Most of the organisms studied showed the reaction to begin within thirty minutes, with dilutions of salt up to 1:50,000, while even the most sensitive showed reduction and formation of characteristic, black compounds in twenty-four hours. The "tellurite reaction" with the colon bacillus is especially rapid, being almost instantaneous, or appearing within a few moments as a brown ring or darkening in all concentrations except the very weak ones.

*S. Belfanti, *Zentralbl. f. Bacteriolog. u. verw. Gebiete*, 1912, Pt. 2, p. 113.

TABLE II.

THE REACTION WITH POTASSIUM TELLURITE OF THE INTESTINAL BACTERIA AND ALLIED ORGANISMS

Organism.	Character of Reaction.	Macroscopical Appearance in Stronger Concentrations 1:10,000—1:20,000.
<i>B. coli communis</i>	Extremely rapid and vigorous	Black, clumpy sediment; liquid above greyish-brown to black and always turbid.
<i>B. typhosus</i>	Moderately rapid, but vigorous	Brown to black, powdery sediment; supernatant liquid always brown and moderately turbid.
<i>B. paratyphosus</i> "A"	Moderately rapid and vigorous	Small, compact brown to black sediment; supernatant liquid dark brown and almost clear.
<i>B. paratyphosus</i> "B"	Strongest action with <i>B. paratyphosus</i> "B" and least with paracolon bacillus	Turbidity least and color strongest with paracolon bacillus.
<i>Bact. (lactis) aërogenes</i> <i>B. (mucosus) capsulatus</i>	Rapid and vigorous	Thick, viscous, black sediment with brownish-black liquid above.
<i>B. enteritidis</i> <i>B. cholerae suis</i>	Rapid and vigorous	Thick, brownish sediment, with liquid above nearly uniform greyish-black to yellow and slightly turbid. Color more nearly brown with <i>B. cholerae suis</i> .
<i>Bact. dysenteriae</i> (Flexner and SBiga)	Slow with Shiga strain, but more rapid with Flexner. Medium intensity in both	Compact, brown to black powdery sediment with liquid above yellowish brown to dark brown and nearly clear.

In Table II, some of the more important organisms under investigation have been grouped in accordance with their reaction to potassium tellurite. From this, it is seen that *B. coli communis* is in a division by itself, as is also *B. typhosus*. A third group is made up of the paratyphoid bacilli and paracolon, while the capsulated bacteria and *Bact. (lactis) aërogenes* comprise a fourth group. The dysentery bacteria fall into another class and *B. cholerae suis* with *B. enteritidis* make up another group.

As may be also noted from the accompanying Plate I, the turbidity and intensity of color are greatest with the colon bacillus, *B. typhosus* coming next. However, the color in case of *B. coli communis* is a greyish-brown while with the typhoid bacillus, it is a dark brown. There are also differences in turbidity and color intensity between *B. paratyphosus* "A" and *B. paratyphosus* "B." Turbidity, color and sediment are all characteristic in the case of *B. enteritidis*, more so than with *B. cholerae suis*. Altogether, the variations both in antiseptic action and the reaction under optimum conditions seem to be sufficient to suggest the experimental use of potassium tellurite as an aid in the differentiation of organisms in the "Colon-Typhoid" group.

C. The Effect of "Tellurization" on the Biochemical Activities of the "Typhoid-Colon" Group.

Microscopical examination of various species of microorganisms seemed to indicate that the process of "tellurization" is essentially a reduction of the potassium tellurite with actual deposition of metallic tellurium, or a black hydrogenated compound within the microbic cell. The appearance of different bacteria so treated has been described in the previous paper on this subject. Aside from altering the morphological characteristics, it seemed as if treatment with tellurite, by introducing foreign substance within the bacterial body, might possibly affect the biological properties of an organism. The bacterial types included in the present study appeared to be well adapted for validating the above hypothesis owing to their biochemical activities.

Dextrose, lactose and saccharose bouillon respectively, and litmus milk were used for determining the bacterial activity before and after treatment with tellurite. The technique consisted in taking a 18-hour culture of the organism to be studied, grown in plain bouillon, and adding to it sufficient potassium tellurite to permit the maximum reduction to take place, with the minimum of germicidal action. For most of the types, a concentration of 1:2500 of tellurite was satisfactory, but for some of the weaker strains, a strength of 1:10,000 gave better results. The "tellu-

TABLE III.

THE INFLUENCE OF POTASSIUM TELLURITE ON THE BACTERIAL FERMENTATION OF DEXTROSE.

Organism.	Action before Tellurization.	Action after Tellurization.
1. <i>B. (mucosus) capsulatus</i>	a, 80	a, 80
2. <i>B. coli communis</i>	a, 40	a, 40
3. <i>Bact. lactisi aerogenes</i>	a, 60	a, 60
4. <i>B. cloacae</i>	a, 40	a, 40
5. <i>B. proteus vulgaris</i>	a, 30	a, 30
6. <i>B. paratyphosus "B"</i>	a, 50	a, 60
7. <i>B. of swine plague</i>	a	a
8. <i>B. enteritidis</i>	a, 40	a, 60
9. <i>B. typhosus</i>	a	a
10. <i>B. paratyphosus "A"</i>	a, 60	a, 60
11. <i>Paracolon Bacillus</i>	a, 40	a, 40
12. <i>B. acidi lactici</i>	a	a
13. <i>B. cholerae sens.</i>	a, 10	a, 10
14. <i>B. pneumoniae (Friedländer)</i>	a	a
15. <i>Bact. dysenteriae (Shiga)</i>	a	a
16. <i>B.icteroides</i>	a	a
17. <i>Bact. dysenteriae (Flexner)</i>	a	a

Note: a = acid, but no gas formation.

Numerals refer to percentage of gas in closed vial.

TABLE IV.

THE INFLUENCE OF POTASSIUM TELLURITE ON THE BACTERIAL FERMENTATION OF LACTOSE.

Organism.	Action before Tellurization.	Action after Tellurization.
1. <i>B. (mucosus) capsulatus</i>	a, 90	a, 90
2. <i>B. coli communis</i>	a, 20	a, 20
3. <i>Bact. (lactis) aërogenes</i>	a, 50	a, 90
4. <i>B. cloacae</i>	a, 40	a, 40
5. <i>Proteus vulgaris</i>	+	+
6. <i>B. paratyphosus</i> "B".....	+	+
7. <i>B. of swine plague</i>	+	+
8. <i>B. enteritidis</i>	+	+
9. <i>B. paratyphosus</i>	+	+
10. <i>B. paratyphosus</i> "A".....	+	+
11. <i>Paracolon bacillus</i>	+	+
12. <i>B. acidi lactici</i>	a, 30	a, 30
13. <i>B. cholerae suis</i>	+	+
14. <i>B. pneumoniae</i> (Friedländer).....	a	a
15. <i>Bact. dysenteriae</i> (Shiga).....	+	+
16. <i>B. icteroides</i>	+	+
17. <i>Bact. dysenteriae</i> (Flexner).....	+	+

Note: a=acid, but no gas formation.

Numerals refer to percentage of gas in closed vial.

+ = growth with neutral reaction and no fermentation.

TABLE V.

THE INFLUENCE OF POTASSIUM TELLURITE ON THE BACTERIAL FERMENTATION OF SACCHAROSE.

Organism.	Action before Tellurization.	Action after Tellurization.
1. <i>B. (mucosus) capsulatus</i>	a, 90	a, 90
2. <i>B. coli communis</i>	a, 90	a, 90
3. <i>Bact. (lactis) aërogenes</i>	a, 50	a, 50
4. <i>B. cloacae</i>	a, 40	a, 40
5. <i>Proteus vulgaris</i>	a, 40	a, 40
6. <i>B. Paratyphosus</i> "B".....	+	+
7. <i>B. of swine plague</i>	+	+
8. <i>B. enteritidis</i>	+	+
9. <i>B. typhosus</i>	a (slight)	a (slight)
10. <i>B. paratyphosus</i> "A".....	+	+
11. <i>Paracolon bacillus</i>	+	+
12. <i>B. acidi lactici</i>	+	+
13. <i>B. cholerae suis</i>	+	+
14. <i>B. pneumoniae</i> (Friedländer).....	+	+
15. <i>Bact. dysenteriae</i> (Shiga).....	+	+
16. <i>B. icteroides</i>	+	+
17. <i>Bact. dysenteriae</i> (Flexner).....	+	+

Note: a=acid, but no gas formation.

Numerals refer to percentage of gas in closed vial.

+ = growth with neutral reaction and no fermentation.

rization" was allowed to go on for 48 hours, after which the supernatant liquid was pipetted off, and two heavy loopfuls of the blackened sediment were used for inoculating the different culture media. Control tubes were similarly inoculated using 96-hour cultures of the organism without tellurite.

TABLE VI.

THE INFLUENCE OF POTASSIUM TELLURITE ON THE BACTERIAL FERMENTATION OF LITMUS MILK.

Organism.	Action before Tellurization.	Action after Tellurization
1 <i>B. (mucosus) capsulatus</i>	a, c	a, c
2 <i>B. coli communis</i>	a, c	a, c
3 <i>Bact. (lactis) aërogenes</i>	a, c	a, c
4 <i>B. cloacae</i>	a, c	a, c
5 <i>B. proteus vulgaris</i>	a, c	a, c
6 <i>B. paratyphosus "B"</i>	alk.	alk.
7 <i>B. of swine plague</i>	a (slight)	alk., then a
8 <i>B. enteritidis</i>	a, then alk.	a, then alk.
9 <i>B. typhosus</i>	a (very slight)	a (very slight)
10 <i>B. paratyphosus "A"</i>	a (slight)	a (slight)
11 <i>Paracolon bacillus</i>	a	a
12 <i>B. acidi lactici</i>	a (slight), c	a (slight), c
13 <i>B. cholerae suis</i>	a, then alk.	alk.
14 <i>B. pneumoniae (Friedländer)</i>	a	a
15 <i>Bact. dysenteriae (Flexner)</i>	alk.	alk.
16 <i>B. icteroides</i>	alk.	alk.
17 <i>Bact. dysenteriae (Flexner)</i>	alk.	alk.

Note: a = acid reaction to the litmus.

alk. = alkaline reaction to the litmus.

c = coagulation.

For the carbohydrate media, inverted vial tubes were used in place of the regular Smith fermentation tubes. Examination was made at the end of 48 hours and again after 96 hours of action. The results obtained with the more important organisms are given in the following tables, using the nomenclature appended in the note with each table, and arranged as in Table I. Acid formation with the sugar media was tested by the coloration imparted to sterile azolitmin solution.

The preceding tables seem to show that treatment with potassium tellurite has but little effect on the biochemical activities of an organism. As might be supposed, the "tellurited" organisms grow more slowly and hence show less fermentative power within the first twenty four hours than do the untreated bacteria. However, at the end of 48 hours, or, better, at the end of 96 hours, some types appear even more vigorous after treatment with tellurite than before. This was shown by increased gas formation in the case of dextrose with *B. enteritidis* and *B. paratyphosus "B,"* and with lactose and *B. lactis aërogenes*. Practically no difference was apparent in the behavior of the "tellurited" organisms and the untreated "controls" both with saccharose and with litmus milk.

CONCLUSIONS.

1. The bacteria of the "Colon-Typhoid Group" show differences in resistance to the antiseptic action of potassium tellurite and in the appearance of their reaction with this salt. These variations are sufficient to suggest the experimental use of potassium tellurite for differential diagnosis in the group.

2. The intensity of bacterial action on potassium tellurite depends upon the individual resistance of the bacterium and the concentration of the salt present. The velocity of reduction of the tellurite is apparently a specific function of an organism, apart from its resistance to antiseptic action. With the colon bacillus, the "tellurite reaction" is almost instantaneous.

3. Treatment with potassium tellurite has practically no influence on the biological characteristics of an organism.

**REPRINTS OF PUBLICATIONS FROM THE RESEARCH
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The present system of collecting reprints of articles published from the Research Laboratory was begun in 1912. Reprints of the following articles published subsequent to that time are available and will be sent upon request. The publications marked (*) are no longer available.

1. On the Administration of Diphtheria Toxin in a Collodion Sac. By E. C. L. Miller. (*Journal of Infectious Diseases*, Vol. 8, January, 1911, pp. 50-65.)

2. A Further Contribution to Our Knowledge of Insecticides—Fumigants. By Chas. T. McClintock, H. C. Hamilton and F. B. Lowe. (*Journal of the American Public Health Association*, Vol. 1, April, 1911, pp. 227-238.)

3. Duboisia Hopwoodii—A Histological Study. By Oliver A. Farwell. (Reprinted from *Merck's Report*, Vol. 20, May 1, 1911.)

*4. Etiology of Canine Distemper. By Newell S. Ferry. (*Journal of Infectious Diseases*, Vol. 8, June, 1911, pp. 399-420.)

*5. The Resistance of Smallpox Vaccine to the Coal-tar Disinfectants. By Chas. T. McClintock and Newell S. Ferry. (*Journal of the American Public Health Association*, Vol. 1, June, 1911, pp. 418-419.)

6. Production of Immunity with Over-Neutralized Diphtheria Toxin. By Chas. T. McClintock and Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Originale, Bd. 59, July 15, 1911, pp. 456-464.)

7. Soaps from Different Glycerides—Their Germicidal and Insecticidal Values Alone and Associated with Active Agents. By H. C. Hamilton. (*Journal of Industrial and Engineering Chemistry*, Vol. 3, August, 1911, pp. 582-584.)

*8. The Sleepy Grass of New Mexico: A Histological Study. By Oliver A. Farwell. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-273.)

*9. Some Observations on the Physiological Action of Sleepy Grass. By A. W. Lescohier. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-275.)

*10. An Investigation of the Depressor Action of Pituitary Extracts. By Carey P. McCord. (*Archives of Internal Medicine*, Vol. 8, November, 1911, pp. 609-620.)

11. The Physiology of the Pituitary Gland and the Actions of Its Extracts. By Carl J. Wiggers. (*American Journal of Medical Sciences*, Vol. 141, April, 1911, pp. 502-515.)

12. A Physiological Investigation of the Treatment of Hemoptysis. By Carl J. Wiggers. (*Archives of Internal Medicine*, Vol. 8, 1911, pp. 17-38.)

13. Notes on Catgut Sterilization: A Preliminary Report. By Willard H. Hutchings. (*Annals of Surgery*, Vol. 54, July, 1911, pp. 693-695.)

14. The Relations of Pyogenic Microorganisms to the Etiology and Treatment of Skin Diseases. By Henry Rockwell Varney. (*Ohio State Medical Journal*, December, 1911.)

15. A Micrococcus with Unusual Characteristics as a Factor in a Resistant Dermatitis Resembling Aene Vulgaris. By Henry Rockwell Varney and L. T. Clark. (*Journal of Cutaneous Diseases*, Vol. 30, February, 1912, pp. 72-78.)

16. Serum Treatment of Hemorrhage and Blood Dyscrasias. By A. W. Lescohier. (*New York Medical Journal*, Vol. 95, February 3, 1912, pp. 223-229.)

*17. Further Studies on the Bacillus Bronchicanis, the Cause of Canine Distemper. By Newell S. Ferry. (*American Veterinary Review*, Vol. 41, April, 1912, pp. 77-79.)

18. The Pharmacopœcial Requirements for Cannabis Sativa. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, March, 1912, pp. 200-203.)

19. The Heart Tonic Unit. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 84, March, 1912, pp. 97-103.)

20. Studies on the Etiology of Equine Influenza. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, April, 1912, pp. 185-197.)

21. A Method for the Bacteriological Standardization of Disinfectants. By Tatsuzo Ohno and H. C. Hamilton. (*American Journal of Public Health*, Vol. 2, May, 1912, pp. 331-338.)

22. Physiological Testing. By E. M. Houghton. (*American Druggist*, July and September, 1911, and January and April, 1912.)

23. Bacillus Bronchisepticus (Bronchicanis): The Cause of Distemper in Dogs and a Similar Disease in Other Animals. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, July, 1912, pp. 376-391.)

24. On Feeding Young Pups the Anterior Lobe of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 30, July, 1912, pp. 352-357.)

25. A Practical Portable Incubator. By Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Original, Bd. 65, Heft 4/5, 1912, pp. 412-413.)

26. Tobacco Extracts: Their Comparative Values as Insecticides. By W. O. Hollister. (*Journal of Economic Entomology*, Vol. 5, June, 1912, pp. 263-267.)

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*37. Studies on the Gonococcus, I. By Carl C. Warden. (*Journal of Infectious Diseases*, Vol. 12, Jan., 1913, pp. 93-105.)

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51. The Treatment of Tetanus. By Charles T. McClintock and William H. Hutchings. (*Journal of Infectious Diseases*, Vol. 13, Sept., 1913, pp. 309-320.)

52. Spirochæta Suis, Its Significance as a Pathogenic Organism. Studies on Hog Cholera. By Walter E. King and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 13, Nov., 1913, pp. 463-498.)

53. Time Recorder for Kymograph Tracings. By Oliver E. Closson. (*Journal of Pharmacology and Experimental Medicine*, Vol. 5, Jan., 1914, pp. 235-238.)

54. U. S. P. Menstrua. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 86, Feb., 1914, pp. 56-61.)

55. Numerical Variations of the White Blood Cells in Mice Inoculated with Transplantable Adenocarcinoma. By F. W. Baeslack. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 20, Heft 5, 1914, pp. 421-435.)

56. A Study of the Germicidal Action of the Ultraviolet Rays. By E. M. Houghton and L. Davis. (*American Journal of Public Health*, Vol. 4, March, 1914, pp. 224-240.)

57. Some Phenomena Involved in the Life History of *Spirochaeta* Suis—Studies on Hog Cholera. By W. E. King and R. H. Drake. (*The Journal of Infectious Diseases*, Vol. 14, March, 1914, pp. 246-250.)

58. The Sterilization of Adrenalin Solutions. By L. W. Rowe. (*American Journal of Pharmacy*, Vol. 86, April, 1914, pp. 145-149.)

59. Infection and Immunity: A Review. By N. S. Ferry, Ph.B., M.D. (*Journal of the American Pharmaceutical Association*, Vol. 3, April and May, 1914.)

60. Disinfection—What Disinfectant is the Most Generally Applicable for Clinical, Surgical and Sanitary Purposes? By H. C. Hamilton. (*Therapeutic Gazette*, Vol. 38, May, 1914, pp. 311-315.)

61. Study of the Bacteriology of the Posterior Nasopharynx in Scarlatina. By N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, pp. 934-935.)

62. Some Experiences with Bacterial Vaccines in Scarlatina. By Guy L. Kiefer, M.D., D.P.H., and N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, p. 936.)

63. A Sero-enzyme Test for Syphilis. By F. W. Baeslack, M.A., M.D. (*The Urologic and Cutaneous Review*, Vol. 18, May, 1914, pp. 234-238.)

64. Bacteriology and Control of Acute Infections in Laboratory Animals. By N. S. Ferry, Ph.B., M.D. (*Journal of Pathology and Bacteriology*, Vol. 18, 1914, pp. 445-455.)

65. The Bacteriological Standardization of Disinfectants. By H. C. Hamilton and Tatsuzo Ohno. (*American Journal of Public Health*, Vol. 4, No. 6, p. 163.)

66. The Pineal Gland in Relation to Somatic, Sexual and Mental Development. By Carey P. McCord, M.D. (*Journal of the American Medical Association*, Vol. 63, July 18, 1914, pp. 232-235.)

67. The Sero-enzyme Test for Syphilis. By F. W. Baeslack, M.D., M.A. (*Journal of the American Medical Association*, Vol. 63, Aug. 15, 1914, pp. 559-563.)

68. A Case of Contagious Broncho-pneumonia Caused by *Bacillus Coli* Communis. By Edwin M. Stanton. (*American Veterinary Review*, Vol. 14, May, 1914, pp. 233-235.)

69. Local Anesthetics—Some Comparative Physiological Reactions. By Oliver E. Closson. (*Journal of the Michigan State Medical Society*, Vol. 13, Oct., 1914, pp. 587-597.)

70. Potassium Tellurite as an Indicator of Microbial Life. By Walter E. King and Lewis Davis. (*American Journal of Public Health*, Vol. 4, Oct., 1914, pp. 917-932.)

71. Further Studies with Reference to Spirochetes Observed in Swine -Studies on Hog Cholera. By Walter E. King, Raymond H. Drake, and Geo. L. Hoffmann. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Vol. 22, 1914, pp. 347-371.)

72. The Pharmacy of Adrenalin. By C. P. Beckwith. (*Journal of the American Pharmaceutical Association*, Vol. 3, November, 1914, pp. 1547-1554.)

73. A Study of the "Tellurite Reaction" with the Colon-typhoid Group and other Organisms. By Lewis Davis, S.M. (*Centralblatt für Bakteriologie Parasitenkunde und Infektionskrankheiten*, 75 Band, 1914, pp. 180-192.)

AN EXPANDING ROOT CANAL FILLING.

BY GEORGE BAILEY HARRIS, D.D.S., S.C.M.

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A root-canal filling, to prove beneficial and successful for what it is intended, must fulfil certain conditions, some of which are as follows:

1. It should be non-contracting, or, if it must contract, the contraction should occur in the center of the filling, and not from the side of the filling toward the center. In other words, the canal filling should adhere to the walls to such an extent that when contraction takes place it will be from the center of the filling toward the walls of the canal.

2. The filling should be mildly antiseptic during its plastic state and have solidification take place slowly.

3. Upon drying, it must not be reduced to a powder, leaving the canal less than half filled.

4. It should be removable.

5. It should be non-irritating.

6. It should prevent the passage of the Roentgen ray.

Were these points applied to the root-canal fillings now being used, we would find one or more of these conditions not fulfilled.

CHLORO-PERCHA.—Chloro-percha, which is used more than any other substance for this purpose, is not immune. It does not prevent the penetration of the rays and it contracts upon drying. It will not contract from the canal wall if the wall is perfectly dry, but it will not adhere to it if there is the least bit of moisture in the canal. This results in an incomplete or partially filled canal.

This is overcome to a certain extent by the use of the gutta-percha point, but the point must go to the end of the root to do this. Radiographers show time and again that the point does not reach anywhere near the apex of the root in a great many cases where such a procedure would seem to be a simple matter. Again, chloro-percha does not readily follow the canal, having a tendency to form a ball when placed in the canal, due to the rapid evaporation of the chloroform. This is overcome to a certain extent by the use of eucalyptol, or like substance; but experiments show

that this is far too irritating to be used in root-canal fillings. The x-rays pass through chloro-percha, making diagnosis by this means extremely difficult.

PARAFFIN.—The case of paraffin is somewhat different. Here we encounter the contraction from the wall toward the center and more or less difficulty in getting it to the apex of the root due to its rapid solidification. It does not hold back the x-rays nor can it be made antiseptic, so that it will remain so after solidification.

THE AUTHOR'S EXPERIMENTS.—Bearing these facts in mind, we set out to produce a substance that would remove as many of these objections as possible, and we believe we have at least partially succeeded. In the first place we have a substance that will expand upon solidifying, instead of contracting. To the liquid is added a "binder" that will not permit its return to a powder upon drying out.

This preparation consists of a powder and a liquid. When these two are mixed together a chemical reaction takes place which results in the expansion of the paste, for it is a paste.

Now, when the paste is stirred rapidly, a contraction results. When the paste in this state is sealed in a pulp chamber it again expands to the chemical reaction which again takes place and which is exaggerated by the rise in temperature. The rise in temperature causes it to take on a semi-solid form, which can be hardened at once by the addition of one drop of another liquid to the canal contents. If this is not added the filling will harden in about two weeks.

At first it was considered necessary to seal up the apex of the root to prevent the expanding paste from passing through the apex, and a great deal of time was devoted to solving this, the final decision being that the best substance for the purpose is a triple salt of tin, made by passing chlorine gas through tin chloride.

After studying the expansion, we found that it could be easily and absolutely controlled; that the expansive power could be eliminated entirely on the one hand or it could be made to expand to such an extent that it would force out a gutta-percha plug when the "normal" or a little above "normal" expansion was placed against resistance equal to that which it would encounter

in a root having a foramen of the diameter of $\frac{1}{2}$ mm. Under these conditions it was found that the expansion was not so great as the tissue pressure or resistance outside of the apex of the root, and that the paste would not pass through unless all expansion possible to put into the paste were used, and it is possible to get considerable more pressure outside the mouth than in, due to a lower temperature at which the experiments may be carried on. For this reason we find that lining the canal and sealing up the apical foramen are unnecessary, except in deciduous teeth, where we believe that an expanding canal filling is both contra-indicated and unnecessary.

There are several ways by which the expansion of this material may be controlled. The most important one is the temperature at which it is mixed and the temperature at which it is introduced into the root canal. If it be mixed at the temperature of the body, there will be neither expansion nor contraction; above that temperature, contraction will take place, and below, expansion. The higher above the normal temperature of the body it is mixed the greater will be the contraction, and the lower the temperature, the greater will be the expansion. So sensitive is it to heat and cold that a fraction of a degree will show a decided difference in its behavior in regard to the expansion or contraction in or out of the canal. Another consideration is the time elapsing between its introduction into the canal and the sealing of the canal. This would affect the expansion only. As soon as it is introduced into the canal, either expansion or contraction begins, unless it is introduced at exactly the temperature of the canal. Expansion will take place along the lines of least resistance, and if the canal is not sealed it will expand out. The more quickly it is sealed in the greater will be the expansion, and where no expansion is desired it is only necessary to leave the canal open for from five to ten minutes, to allow all expansion to take place, then sealing it up. There will then be no pressure in the canal and the filling will not be dense. Should it then be found desirous to expand it, or to condense it a little, all that would be required would be the addition of one or two drops of liquid introduced into the canal at a low temperature and quickly sealed with cement. Expansion and condensation will then take place in a very few minutes. The density of the filling will be

governed by the quantity of the powder added to the liquid as well as the temperature. The more powder used, the slower will be the expansion, the denser the filling and the less the expansion. However, by the addition of all the powder possible to add to the liquid, the expansion cannot be prevented entirely.

TUBE EXPERIMENTS WITH EXPANDING ROOT FILLING.—Where contraction takes place it contracts in the center. This is shown in Fig. 1, tube 2. This tube was filled up to F and then gently warmed until it expanded to R. It was then allowed to cool. Contraction occurred, not at the side of the tube, but in the center of the filling downward and toward the sides of the tube to N, the remainder being solid. The distance from F to N

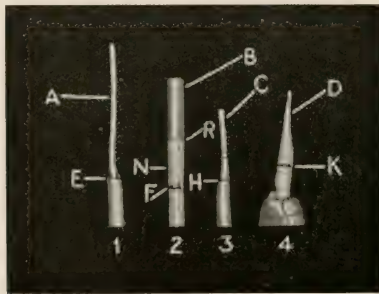


Fig. 1.

represents the extent of normal expansion contained in the paste at the temperature it was mixed and at room temperature. An alcohol flame was then applied below F and expansion again took place, expanding to B. Were this temperature maintained until solidification occurred no contraction would result, but as the flame was withdrawn as soon as B was reached, contraction took place, finally returning to the same level where it was before the alcohol flame was applied. As this tube is of the same diameter throughout its full length, the paste was expanded more than its own volume and maintained it throughout solidification. To show how sensitive it is to thermal changes, we will consider tubes 3 and 4 in the same illustration. In these two cases the paste was

prepared at room temperature and put into the two tubes at the same temperature. No heat was applied from outside sources, *i.e.*, all the heat involved in producing the expansion was the result of the chemical reaction which took place as a result of the powder and liquid being mixed together. As a matter of fact, the rise in temperature is so slight that it is detected with difficulty; yet this small change is sufficient to cause the mixture to expand from H to C in No. 3 and from K to D in No. 4.

Were the paste allowed to dry and then be pulverized and have the liquid again added, while it would expand under certain conditions, its extreme sensitiveness would be lost, but there would still remain sufficient expansive powder to fill a reasonable space. Its response, however, would be slower.

In the experiments shown so far, all the tubes have been open at one end, allowing the air between the paste and the end of the tube to escape as expansion took place. This condition does not exist in the root canal. What then becomes of this air in the canal? Is it forced beyond the apex of the root into the surrounding tissues, or will it prevent expansion? It is not forced beyond the apex, because the same amount of pressure preventing the paste to expand beyond the apex also prevents the air from passing beyond. Again, it does not prevent expansion.

Tube No. 1 shows the expansion taking place in a sealed tube quite as readily as it does in the open tubes. This tube was filled and the filling expanded under the same conditions as were Nos. 3 and 4, the air offering no resistance whatever. The air is taken up by the paste as it expands, filling the microscopic spaces produced when the molecules swell during the expansion, and there it remains. Whether any of the air is chemically taken up by the paste or not has not yet been determined, but it likely is not.

POSSIBILITY OF REMOVAL OF ROOT FILLING.—The next consideration is its removal from the canal, should this become necessary. Let us suppose that complete solidification has taken place and that the filling is perfectly hard. Its removal is an easy matter. Simply open up into the pulp chamber and then chill the tooth with ice or a cold blast of air and fill the canal with the expanding liquid, previously chilled. This will be rapidly taken up by the filling when another drop is added, keeping both the liquid and tooth as cold as possible. Remove the ice or cold blast

and either apply a warm blast or allow it to be warmed from the tissues. In a few minutes it will again expand, following the line of least resistance, which in this case will be toward the crown of the tooth. In other words, it will expand outwardly. Additional liquid can then be carried on a broach and the entire filling removed without any danger of forcing it through the foramen and without the slightest discomfort to the patient. The operation is also very quickly done.

RESISTANCE TO X-RAY.—An important feature which this material possesses is its ability to stop the Roentgen ray. This was overlooked in the original powder and was suggested to me by Dr. Giffin, of Detroit, who pointed out its extreme importance, and after considerable experimenting we were able to add this factor to the material without interfering with any of the other points. Supposing that we fill a root canal, or, better, fill two root canals in a molar tooth, but are unable to get into the third for some reason. The chances are that this third root will be completely filled by the expanding, but we would not be sure. An x-ray would tell this at once, and in case it was not completely filled all that would be required would be the addition of a little liquid, resulting in a continued expansion. This operation would be continued until the radiograph showed all the canals completely filled. This can be done in third molars by simply filling the pulp chamber half full of the paste, filling the balance with cement and allowing the expansion to fill the canals.

REPRINTS OF PUBLICATIONS FROM THE RESEARCH
LABORATORY, PARKE, DAVIS & CO.,
DETROIT, MICH.

The present system of collecting reprints of articles published from the Research Laboratory was begun in 1912. Reprints of the following articles published subsequent to that time are available and will be sent upon request. The publications marked (*) are no longer available.

1. On the Administration of Diphtheria Toxin in a Collodion Sac. By E. C. L. Miller. (*Journal of Infectious Diseases*, Vol. 8, January, 1911, pp. 50-65.)

2. A Further Contribution to Our Knowledge of Insecticides—Fumigants. By Chas. T. McClintock, H. C. Hamilton and F. B. Lowe. (*Journal of the American Public Health Association*, Vol. 1, April, 1911, pp. 227-238.)

3. *Duboisia Hopwoodii*—A Histological Study. By Oliver A. Farwell. (Reprinted from *Merck's Report*, Vol. 20, May 1, 1911.)

*4. Etiology of Canine Distemper. By Newell S. Ferry. (*Journal of Infectious Diseases*, Vol. 8, June, 1911, pp. 399-420.)

*5. The Resistance of Smallpox Vaccine to the Coal-tar Disinfectants. By Chas. T. McClintock and Newell S. Ferry. (*Journal of the American Public Health Association*, Vol. 1, June, 1911, pp. 418-419.)

6. Production of Immunity with Over-Neutralized Diphtheria Toxin. By Chas. T. McClintock and Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Originale, Bd. 59, July 15, 1911, pp. 456-464.)

7. Soaps from Different Glycerides—Their Germicidal and Insecticidal Values Alone and Associated with Active Agents. By H. C. Hamilton. (*Journal of Industrial and Engineering Chemistry*, Vol. 3, August, 1911, pp. 582-584.)

*8. The Sleepy Grass of New Mexico: A Histological Study. By Oliver A. Farwell. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-273.)

*9. Some Observations on the Physiological Action of Sleepy Grass. By A. W. Lescohier. (*Merck's Report*, Vol. 20, October, 1911, pp. 271-275.)

*10. An Investigation of the Depressor Action of Pituitary Extracts. By Carey P. McCord. (*Archives of Internal Medicine*, Vol. 8, November, 1911, pp. 609-620.)

11. The Physiology of the Pituitary Gland and the Actions of Its Extracts. By Carl J. Wiggers. (*American Journal of Medical Sciences*, Vol. 141, April, 1911, pp. 502-515.)

12. A Physiological Investigation of the Treatment of Hemoptysis. By Carl J. Wiggers. (*Archives of Internal Medicine*, Vol. 8, 1911, pp. 17-38.)

13. Notes on Catgut Sterilization: A Preliminary Report. By Willard H. Hutchings. (*Annals of Surgery*, Vol. 54, July, 1911, pp. 693-695.)

14. The Relations of Pyogenic Microorganisms to the Etiology and Treatment of Skin Diseases. By Henry Rockwell Varney. (*Ohio State Medical Journal*, December, 1911.)

15. A Micrococcus with Unusual Characteristics as a Factor in a Resistant Dermatitis Resembling Acne Vulgaris. By Henry Rockwell Varney and L. T. Clark. (*Journal of Cutaneous Diseases*, Vol. 30, February, 1912, pp. 72-78.)

16. Serum Treatment of Hemorrhage and Blood Dyscrasias. By A. W. Lescohier. (*New York Medical Journal*, Vol. 95, February 3, 1912, pp. 223-229.)

*17. Further Studies on the *Bacillus Bronchicanis*, the Cause of Canine Distemper. By Newell S. Ferry. (*American Veterinary Review*, Vol. 41, April, 1912, pp. 77-79.)

18. The Pharmacopœial Requirements for *Cannabis Sativa*. By H. C. Hamilton. (*Journal of the American Pharmaceutical Association*, Vol. 1, March, 1912, pp. 200-203.)

19. The Heart Tonic Unit. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 84, March, 1912, pp. 97-103.)

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21. A Method for the Bacteriological Standardization of Disinfectants. By Tatsuzo Ohno and H. C. Hamilton. (*American Journal of Public Health*, Vol. 2, May, 1912, pp. 331-338.)

22. Physiological Testing. By E. M. Houghton. (*American Drug-gist*, July and September, 1911, and January and April, 1912.)

23. *Bacillus Bronchisepticus* (*Bronchicanis*): The Cause of Distemper in Dogs and a Similar Disease in Other Animals. By Newell S. Ferry. (*Veterinary Journal* (London), Vol. 19, July, 1912, pp. 376-391.)

24. On Feeding Young Pups the Anterior Lobe of the Pituitary Gland. By T. B. Aldrich. (*American Journal of Physiology*, Vol. 30, July, 1912, pp. 352-357.)

25. A Practical Portable Incubator. By Newell S. Ferry. (*Abdruck Aus Dem Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Abt. 1, Original, Bd. 65, Heft 4/5, 1912, pp. 412-413.)

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50. A Comparative Study of Antigens for the Wassermann Reaction. By H. R. Varney and F. W. Baeslack. (*Journal of the American Medical Association*, Vol. 61, Sept. 6, 1913, pp. 754-757.)

51. The Treatment of Tetanus. By Charles T. McClintock and Willard H. Hutchings. (*Journal of Infectious Diseases*, Vol. 13, Sept., 1913, pp. 309-320.)

52. *Spirochæta Suis*, Its Significance as a Pathogenic Organism, Studies on Hog Chlorea. By Walter E. King and George L. Hoffmann. (*Journal of Infectious Diseases*, Vol. 13, Nov., 1913, pp. 463-498.)

53. Time Recorder for Kymograph Tracings. By Oliver E. Closson. (*Journal of Pharmacology and Experimental Medicine*, Vol. 5, Jan., 1914, pp. 235-238.)
54. U. S. P. Menstrua. By H. C. Hamilton. (*American Journal of Pharmacy*, Vol. 86, Feb., 1914, pp. 56-61.)
55. Numerical Variations of the White Blood Cells in Mice Inoculated with Transplantable Adenocarcinoma. By F. W. Baeslack. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Bd. 20, Heft 5, 1914, pp. 421-435.)
56. A Study of the Germicidal Action of the Ultraviolet Rays. By E. M. Houghton and L. Davis. (*American Journal of Public Health*, Vol. 4, March, 1914, pp. 224-240.)
57. Some Phenomena Involved in the Life History of Spirochæta Suis—Studies on Hog Cholera. By W. E. King and R. H. Drake. (*The Journal of Infectious Diseases*, Vol. 14, March, 1914, pp. 246-250.)
58. The Sterilization of Adrenalin Solutions. By L. W. Rowe. (*American Journal of Pharmacy*, Vol. 86, April, 1914, pp. 145-149.)
59. Infection and Immunity: A Review. By N. S. Ferry, Ph.B., M.D. (*Journal of the American Pharmaceutical Association*, Vol. 3, April and May, 1914.)
60. Disinfection—What Disinfectant is the Most Generally Applicable for Clinical, Surgical and Sanitary Purposes? By H. C. Hamilton. (*Therapeutic Gazette*, Vol. 38, May, 1914, pp. 311-315.)
61. Study of the Bacteriology of the Posterior Nasopharynx in Scarlatina. By N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, pp. 934-935.)
62. Some Experiences with Bacterial Vaccines in Scarlatina. By Guy L. Kiefer, M.D., D.P.H., and N. S. Ferry, M.D. (*Medical Record*, Vol. 85, May 23, 1914, p. 936.)
63. A Sero-enzyme Test for Syphilis. By F. W. Baeslack, M.A., M.D. (*The Urologic and Cutaneous Review*, Vol. 18, May, 1914, pp. 234-238.)
64. Bacteriology and Control of Acute Infections in Laboratory Animals. By N. S. Ferry, Ph.B., M.D. (*Journal of Pathology and Bacteriology*, Vol. 18, 1914, pp. 445-455.)
65. The Bacteriological Standardization of Disinfectants. By H. C. Hamilton and Tatsuzo Ohno. (*American Journal of Public Health*, Vol. 4, No. 6, p. 163.)
66. The Pineal Gland in Relation to Somatic, Sexual and Mental Development. By Carey P. McCord, M.D. (*Journal of the American Medical Association*, Vol. 63, July 18, 1914, pp. 232-235.)
67. The Sero-enzyme Test for Syphilis. By F. W. Baeslack, M.D., M.A. (*Journal of the American Medical Association*, Vol. 63, Aug. 15, 1914, pp. 559-563.)
68. A Case of Contagious Broncho-pneumonia Caused by Bacillus Coli Communis. By Edwin M. Stanton. (*American Veterinary Review*, Vol. 14, May, 1914, pp. 233-235.)
69. Local Anesthetics—Some Comparative Physiological Reactions. By Oliver E. Closson. (*Journal of the Michigan State Medical Society*, Vol. 13, Oct., 1914, pp. 587-597.)
70. Potassium Tellurite as an Indicator of Microbial Life. By Walter E. King and Lewis Davis. (*American Journal of Public Health*, Vol. 4, Oct., 1914, pp. 917-932.)

71. Further Studies with Reference to Spirochetes Observed in Swine—Studies on Hog Cholera. By Walter E. King, Raymond H. Drake, and Geo. L. Hoffmann. (*Zeitschrift für Immunitätsforschung und Experimentelle Therapie*, Vol. 22, 1914, pp. 347-371.)

72. The Pharmacy of Adrenalin. By C. P. Beckwith. (*Journal of the American Pharmaceutical Association*, Vol. 3, November, 1914, pp. 1547-1554.)

73. A Study of the "Tellurite Reaction" with the Colon-typhoid Group and other Organisms. By Lewis Davis, S.M. (*Centralblatt für Bakteriologie Parasitenkunde und Infektionskrankheiten*, 75 Band, 1914, pp. 180-192.)

74. An Expanding Root Canal Filling. By George Bailey Harris, D.D.S., Sc.M. (*Items of Interest*, Vol. 36, Dec., 1914, pp. 881-886.)

